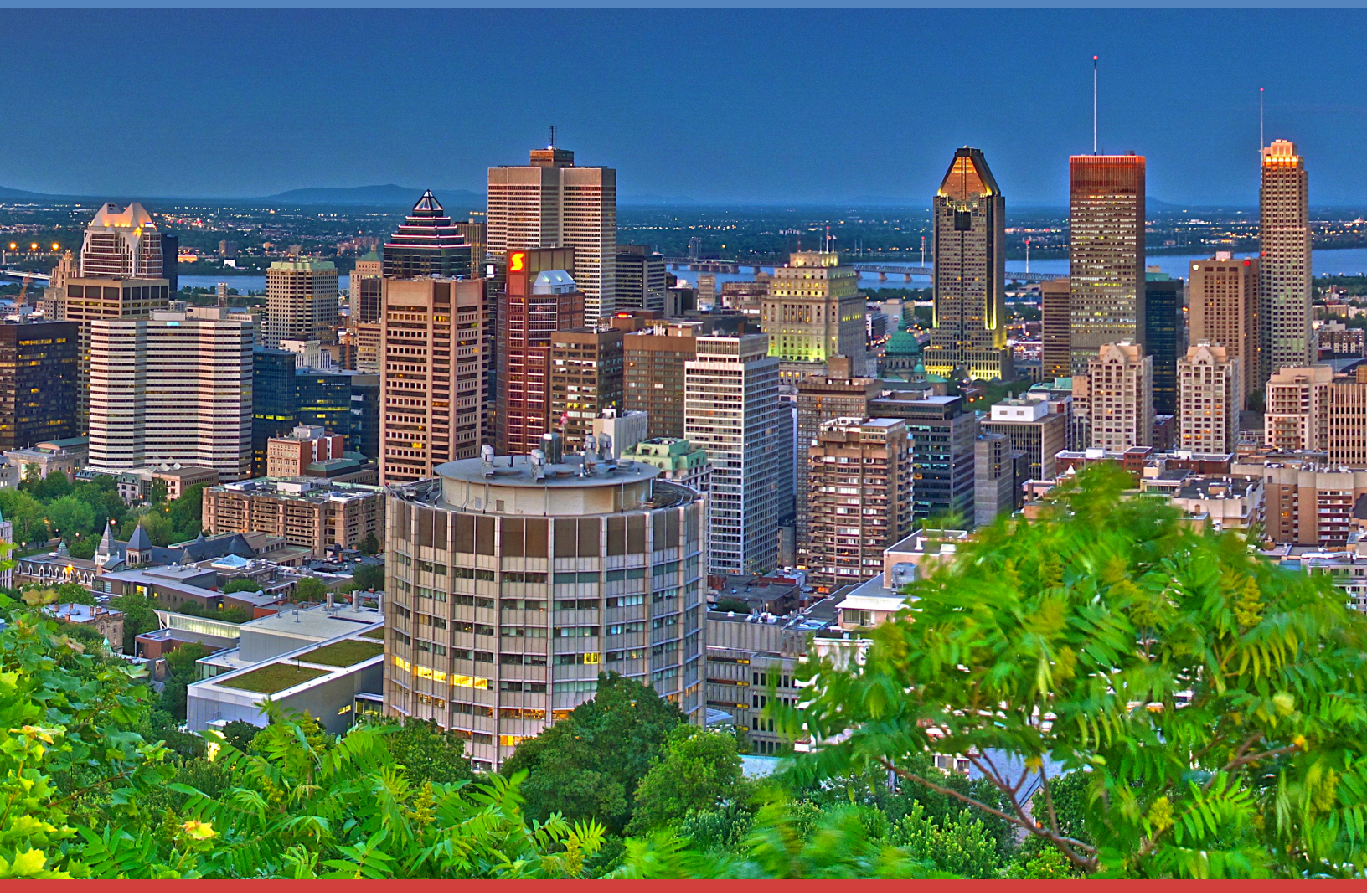
Canadian Connective Tissue Society

May 29 - 31, 2019



Hôpitaux Shriners
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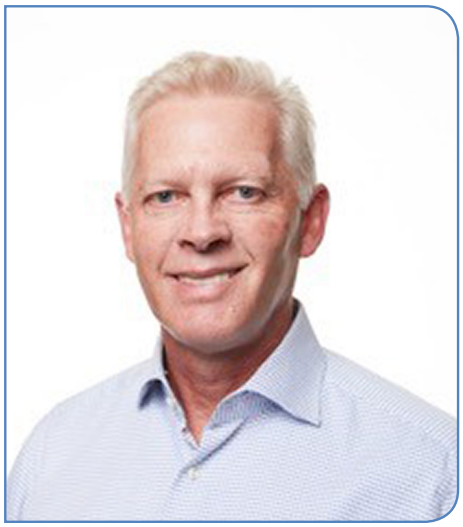
Canada

Word from the President



Colleagues and CCTC attendees, welcome to Montreal and the Canadian Connective Tissue Conference for 2019 (CCTC 2019). The CCTC has been an annual meeting held since 1994 making this year the 25th anniversary. It is the flagship vehicle for the Society, acting as a convergence point for leading investigators and trainees to share and discuss current/dynamic research in multiple aspects of connective tissue. Connective tissue integrity is critical in organ structure/function of bone, cartilage, vasculature, skin and lung, which all interact with inflammation/immunity, metabolism, tissue engineering, disease and environment.

The CCTC connects researchers across the country with conference themes including fundamental biology, genetics and molecular biology, repair and regeneration, tissue fibrosis, health and development, and other topics in context of connective tissue. A major goal of the Society and the CCTC is to assist in motivation and integration of trainees and young investigators in connective tissue research, which will move forward in an increasingly interdisciplinary manner. We encourage high participation by trainees and young scientists at the CCTC.

The organizing committee co-chairs for CCTC 2019 are Dr. Derek Rosenzweig and Dr. Svetlana Komarova, both from McGill University. They have put together an excellent and intriguing program that will be held at Shriners Hospitals for Children — Canada this year. On behalf of the board of the Canadian Connective Tissue Society (CCTS), a warm welcome to Montreal and this year's get together.

Regards,

Carl D. Richards, PhD
President, Canadian Connective Tissue Society
Professor and Director
McMaster Immunology Research Centre

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Dr. Bettina Willie
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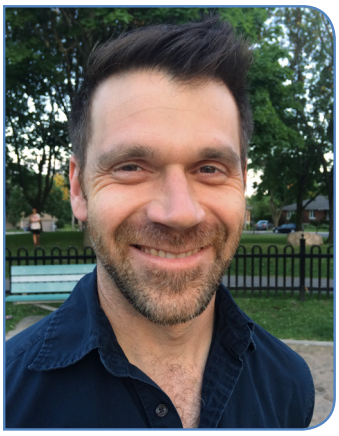
Welcome from the Co-Chairs!

Prof. Svetlana Komarova
McGill University



Prof. Derek Rosenzweig
McGill University

On behalf of the Canadian Connective Tissue Society, we are pleased to invite you to the 25th Annual Canadian Connective Tissue Conference, 2019. The goal of this conference is to fill the gaps in current scientific and clinical understanding of connective tissues in both health and disease through communication of health research evidence between Canadian basic scientists, clinicians, and industrial partners. Here, we provide a forum for scientists, clinicians, students, and other individuals representing the broad scope of connective tissue diseases that affect bones, muscles, skin and joints, to come together and exchange knowledge, to collectively move forward in the field of connective tissue research.

This year's meeting will take place from May 29 through May 31 at Shriners Hospitals for Children — Canada in Montreal, QC and will showcase leading scientists and clinicians working on several themes in connective tissue development and remodeling in health and disease. There will also be a gala dinner on the 30th at the McGill University Faculty Club.

Some of the invited speakers for CCTC this year include key note speaker Dr. René St-Arnaud, Director of Shriners Canada Research Centre and Francis Glorieux Chair, Dr. Kjetil Ask, Associate Professor in the Department of Medicine at McMaster University, Dr. Philippe Campeau, Deputy Chief of Musculoskeletal Diseases and Rehabilitation Axis, CHU Sainte-Justine Research, University of Montreal, Dr. Colin Crist, Marjorie and Gerald Bronfman Research Chair in Muscle Stem Cell Research at McGill University, Dr. Luda Diatchenko, Canada Excellence Research Chair (CERC) in Human Pain Genetics, McGill University, Dr. Julie Fradette, Centre de Recherche en Organogénèse Expérimentale de l'Université Laval/ LOEX, Dr. Ed Harvey, Michal and Renata Hornstein Chair in Surgical Excellence and Leader of Injury, Repair, and Recovery Program at RI MUHC, Dr. David Holdsworth Scientific Director of the Bone and Joint Institute and Scientist in the Imaging group at the Robarts Research Institute and Professor in the Departments of Surgery and Medical Biophysics in the Schulich School of Medicine and Dentistry, at Western University, Dr. Saija Kontulainen, Professor in the College of Kinesiology, University of Saskatchewan, Dr. Roman Krawetz, The McCaig Institute for Bone and Joint Health, University of Calgary, Dr Sylvie Ricard-Blum Professor of Biochemistry at the University of Lyon, and Deputy Director of the Interdisciplinary Sciences - Health doctoral school, and Dr. Lidan You, Associate Professor at the Department of Industrial and Mechanical Engineering, University of Toronto.

We are very excited for this year's program, and we look forward to your attendance!

Program Day 1 & 2

Day 1	
19:00	Registration opens
19:00 - 21:30	Welcome Reception, Thomson House, McGill Campus
Day 2 **Shriners, 2nd floor conference room**	
7:45 – 8:30	**Breakfast**
8:30 – 8:45	Welcome Remarks
SESSION I	**Connective tissue development in health and disease** **Chairs: Morris Manolson and Annie Levasseur**
8:45 – 9:15	Keynote speaker **René St-Arnaud**, Shriners Hospitals for Children — Canada 24,25-dihydroxyvitamin D and fracture repair: from mechanisms to clinical trial
9:15 – 10:05	Selected abstracts for oral presentation
9:15-9:25	**Rongmo Zhang** R. Zhang, H. Kumra, D.P. Reinhardt Involvement of microRNAs in aortic aneurysm formation in Marfan syndrome
9:25-9:35	**Byron Chan** B. Chan, J. Parreno, M. Glogauer, Y. Wang, R. Kandel The actin binding protein adseverin is involved in regulating articular chondrocyte phenotype
9:35-9:45	**Elizabeth Zimmermann** E. Zimmermann, K. Tiedemann, C. Julien, S.V. Komarova, D.P. Reinhardt, B.M. Willie Examining tissue composition, whole-bone morphology and mechanical behavior in Marfan Syndrome
9:45-9:55	**K.J. Aitken** K.J. Aitken, J.X. Jiang, M. Sidler, A. Schroder, A. Ahmed, P. Delgado-Olguin, D.J. Bagli AKT pathway in bladder obstruction and matrix-induced smooth muscle phenotype
9:55-10:05	**Omar Al Rifai** O. Al Rifai, R. Essalmani, J. Creemers, N. Seidah, M. Ferron Regulation of FGF23 processing and bone mass accrual by the proprotein convertase furin
10:05 – 10:30	Invited speaker **Colin Crist**, Lady Davis Institute Translational control of skeletal muscle stem cell quiescence and self-renewal
10:30 – 10:45	**Coffee break**
SESSION II	**Genetics and molecular biology of connective tissue disorders** **Chairs: Dieter Reinhardt and Joanne Tang**
10:45 – 11:10	Invited speaker **Luda Diatchenko**, McGill University Understanding chronic pain through genomics and transcriptomics
11:10-11:50	Selected abstracts for oral presentation
11:10-11:20	**Hélène Mathieu** H. Mathieu, S. Parent, V. Cunin, A. Spataru, S. Ehresmann, J. Rousseau, TT. Nguyen, V. Saillour, S. Barchi, J. Joncas, J. Antonio, P. Shunmoogum, A. Child, P. Campeau, F. Moldovan Genetic analysis of familial AIS: Implication of ciliary genes
11:20-11:30	**Gilles Tremblay** J.-F. Denis, T. Gruosso, Gilles Tremblay, I. Tikhomirov, M. O'Connor-McCourt Development of AVID200, a novel TGF-beta 1 & 3 inhibitor for the treatment of fibrotic diseases
11:30-11:40	**Elizabeth Stephens** E. Stephens, M. Roy, M. Bisson, S. Roux Dysregulated microRNA expression in osteoclasts from Paget's disease of bone
11:40-11:50	**Mariya Stavnichuk** M. Stavnichuk, S.V. Komarova Progressive bone gain in G6b-B knock-out mice

11:50-12:15	Invited speaker **Philippe Campeau**, The Centre hospitalier universitaire Sainte-Justine Understanding growth plate disorders to better treat them
12:15-13:45	**Lunch and Poster Presentation Session 1**
12:15-13:45	CCTS Board Meeting (board members only)
SESSION III	**Connective tissue repair and regeneration Chairs: Lisbet Haglund and Kyle MacDonald**
13:45 – 14:10	Invited speaker **Roman Krawetz**, University of Calgary The role of cell cycle activation in endogenous cartilage regeneration
14:10 – 15:00	Selected abstracts for oral presentation
14:10-14:20	**Rayan Fairag** R. Fairag, D.H. Rosenzweig, J.L. Ramirez-Garcialuna, M.H. Weber, L. Haglund Feasibility of 3D-printed polylactic acid (PLA) scaffolds in promoting bone-like matrix deposition in vitro
14:20-14:30	**Marieanne Comeau-Gauthier** M. Comeau-Gauthier, M. Tarchala, J.L. Ramirez-Garcia Luna, E.J. Harvey, G. Merle Accelerating fracture repair by activating Wnt/β-catenin signaling pathway via Tideglusib release
14:30-14:40	**Nan Wu** N. Wu, S. Wang, Y. Xie, W. Du, X. Li, J. Xu, L. Zhou, K. Zeng, F. Awan, E. Eshaghi, J Lyu, B. Yang YAP circular RNA, circYAP, attenuates cardiac fibrosis via binding with tropomyosin-4 and gammaactin
14:40-14:50	**Elie Akoury** E. Akoury, P. Ahangar, A.S. Ramirez Garcia Luna, M.H. Weber, D.H. Rosenzweig Low-dose zoledronate for local delivery to patient-derived spinal bone metastasis secondary to lung cancer
14:50-15:00	**Sarah Hedtrich** K. Hörst, L. van den Broek, S. Gibbs, U. v. Fritschen, S. Hedtrich Myofibroblast reprogramming in hypertrophic scars is mediated by adipocytes via BMPs
15:00 – 15:25	Invited speaker **Julie Fradette**, Laval University Engineering natural organ specific 3D models for in vitro and pre-clinical studies
15:25-15:40	**Coffee Break**
15:40-16:10	CCTS Business Meeting/General Assembly (all CCTC participants)
SESSION IV	**Short communications Chairs: Craig Simmons and Rosalie Fortin-Trahan**
16:10-16:20	Industrial talk Cellink Bioprinting: materials methods and motivations
16:20 – 17:00	Selected abstracts for short oral presentations **Daniel Bisson**, P. Lama, D.H. Rosenzweig, E. Krock, J.A. Ouellet, L. Haglund. Role of Toll-like receptors in degenerating scoliotic facet joints **Poulami Datta**, S. Nakamura, E. Rossomacha, H. Endisha, C. Younan, K.H. Borada, K. Perry, N.N. Mahomed, R. Gandhi, J.S. Rockel, M. Kapoor. Attenuation of surgically-induced osteoarthritis (OA) by inhibition of autotaxin **Iris Boraschi-Diaz**, F. Rauch. Combination treatment of novel ActRIIB ligand Trap and Zolendronate improves bone-muscle proprieties in osteogenesis imperfecta **Chrisanne D'souza**, N. Mikolajewicz, S.V. Komarova. Age-associated changes in purinergic mechanotransduction **Salem Werdyani**, M. Liu, A. Furey, E. Randell, P. Rahman, G. Zhai. Endotypes of primary osteoarthritis identified by a metabolomics approach **Alice Bouchard**, B. Willie. Load-induced bone formation in mice is not affected by time of loading **Magdalena Wojtas**, M.H. Yu, E.D. Sone. The effect of cross-linking and net charge on collagen mineralization **Fei Geng**, B. Che, C. Patel, Y. Bai. The regulation of autophagy via YAP signaling in systemic sclerosis **Hana Hakami**, V. Moulin, N. Lamarche-Vane, D.P. Reinhardt. Fibulin-4 and latent transforming growth factor-β binding protein-4 in wound healing **Patrick Murphy**, A. McClennan, L. Hoffman. Characterization of Wilms' tumour 1 (WT1) as a fibrotic biomarker for Duchenne muscular dystrophy
18:30-22:00:	**Gala dinner and social, McGill University Faculty Club, McGill Campus**

Program Day 3

Day 3 Shriners, 2nd floor conference room	
7:45 – 8:30	**Breakfast**
SESSION V	**Fundamentals of extracellular matrix biology Chairs: Mari Kaartinen and Brian Wu**
8:30 – 8:55	Invited speaker **Sylvie Ricard-Blum**, University of Lyon Insights into the structure, interactions and functions of a matricryptin, the pro-peptide of lysyl oxidase
8:55 – 9:45	Selected abstracts for oral presentation
8:55-9:05	**Nina Noskovicova** N. Noskovicova, S. Van Putten, A. Koehler, S. Boo, D. Griggs, P. Ruminski, R. Bank and B. Hinz Inhibiting fibrotic encapsulation of body implants by targeting mechanical activation of profibrotic TGF-ß1
9:05-9:15	**Venket Ravivarma** K. Ho, V. Ravivarma, A.M. Pena Diaz, D.B. O'Gorman Cytokines enhance interactions between TßL1, TßLR1, Small Ubiquitin-like MOdifiers (SUMOs) and ß-catenin in palmar fascia fibroblasts
9:15-9:25	**Helal Endisha** H. Endisha, P. Datta, A. Sharma, S. Nakamura, E. Rossomacha, C. Younan, G. Tavallaee, R. Gandhi, M. Kapoor Elucidating the functional role of miR-34a in osteoarthritis pathogenesis – Involvement in obesity and osteoarthritis
9:25-9:35	**Nuno Coelho** N. Coelho, A. Wang, P. Petrovic, Y. Wang, W. Lee, C.A. McCulloch Myosin phosphatase Rho-interacting protein regulates DDR1-myosin II interactions and collagen contraction
9:35-9:45	**Ahmed Alshaer** A. Alshaer, O. Salem, M.P. Grant, L.M. Epure, O.L. Huk, J. Antoniou, F. Mwale Free calcium regulates the expression of proteoglycan and collagen in human cartilage
9:45 – 10:10	Invited speaker **Kjetil Ask**, McMaster University Cellular and molecular phenotyping and imaging pipeline for target characterization in archived biobank samples
10:10 – 10:25	**Coffee break**
SESSION VI	**Clinical and translational research in connective tissue biology Chairs: Sophie Roux and Pouyan Ahangar**
10:25 – 10:50	Invited speaker **Edward Harvey**, McGill University Taking temperature modulation from the bedside to the bench
10:50-11:30	Selected abstracts for oral presentation
10:50-11:00	**Leah Ferrie** L. Ferrie, P. Premnath, B. Besler, L. Larijani, D. Rancourt, M. Underhill, N. Duncan, R. Krawetz The role of exogenous and endogenous stem cells and biomaterials in bone fracture healing
11:00-11:10	**Jose Ramirez-Garcialuna** J. Ramirez-Garcialuna, O.O. Olasubulumi, D. Rosenzweig, J.E. Henderson, P.A. Martineau Enhanced bone repair after fracture priming
11:10-11:20	**Myron R. Szewczuk** M.R. Szewczuk, S. Haq, V. Samuel, F. Haxho, R. Akasov, M. Leko, S.V. Burov, E. Markvicheva Aberrant sialoglycan patterns facilitate 3D multicellular spheroid and xenograft tumor formation
11:20-11:30	**Hosni Cherif** H. Cherif, D. Bisson, S. Kocabas, L. Haglund Natural senolytics to relief back pain.
11:30-11:55	Invited speaker **Saija Kontulainen**, University of Saskatchewan Strong bones across the lifespan: role of mechanical loading

Day 3

Shriners, 2nd floor conference room

12:00-13:30	**Lunch and Poster Presentation Session 3**
SESSION VII	**Imaging, genetics and high-throughput screening of connective tissues** **Chairs: Fackson Mwale and Alexandria DeCarl**
13:30 – 13:55	Invited speaker **David Holdsworth**, Western University Pre-clinical and clinical imaging of the musculoskeletal system
13:55 – 14:35	Selected abstracts for oral presentation
13:55-14:05	**Amanda Ali** A. Ali, R. Gandhi, P. Potla, K. Shestopaloff, S. Lively, K. Perry, C.T. Appleton, M. Kapoor Known and novel circulating microRNAs are uniquely expressed in knee osteoarthritis
14:05-14:15	**Annie Levasseur** A. Levasseur, F. Guillaume, H-L. Ploeg, Y. Petit Can micro-computed tomograhy detect bone adaptations from ex vivo culture?
14:15-14:25	**Hadla Hariri** H. Hariri, W. Addison, M Pellicelli, R. St-Arnaud Usp53, a PTH target regulating bone turnover and mesenchymal stem cell differentiation
14:25-14:35	**Joanne Tang** J. Tang, A. McClennan, J. Hadway, H. Smailovic, M. Fox, U. Anazodo, L. Hoffman The non-invasive modelling of body-wide inflammation in Duchenne muscular dystrophy
14:35 – 15:00	Invited speaker **Lidan You**, University of Toronto Bone cell crosstalk under mechanical loading
15:00-15:30	**Awards Presentation & Concluding Remarks**

Invited Speakers

Dr. René St-Arnaud, PhD, is the inaugural Francis Glorieux Professor in Pediatric Musculoskeletal Research at McGill University. He is currently Director of Research at Shriners Hospitals for Children – Canada and cross-appointed as a tenured Professor of Surgery at McGill. His research focuses on vitamin D metabolism and the control of gene expression in osteoblasts. His contributions to the understanding of bone cell differentiation and function have been recognized through several distinctions, most recently the C.P. Leblond Career Award. Dr. St-Arnaud has a total of 141 peer-reviewed publications, 40 book chapters or conference proceedings and holds seven medical invention patents.

Dr. Colin Crist is an Associate Professor in the Department of Human Genetics at McGill University and Principal Investigator at the Lady Davis Institute for Medical Research. He obtained his PhD from the University of Tokyo and was a post-doctoral associate in the Department of Stem Cell and Developmental Biology at the Institut Pasteur (Paris, France) where he made contributions to our understanding of post-transcriptional regulation of gene expression within the context of mouse skeletal muscle development and regeneration. His laboratory investigates the molecular biology underlying muscle stem cell activity, with the rationale that deepening our understanding of how muscle stem cells develop and function will be key to realizing regenerative medicine based approaches to treating muscle disease.

Dr. Luda Diatchenko, MD, PhD is a Canada Excellence Research Chair in Human Pain Genetics, and professor in the Faculty of Medicine, Department of Anesthesia, and Faculty of Dentistry, at McGill University. She earned her MD and PhD in the field of Molecular Biology from the Russian State Medical University. Dr. Diatchenko started her career in industry as a Leader of the RNA Expression Group at Clontech, and subsequently, Director of Gene Discovery at Attagene. During this time, Dr. Diatchenko was actively involved in the development of several widely-used and widely-cited molecular tools for the analysis of gene expression and regulation. Dr. Diatchenko's academic career started at 2000 in the Center for Neurosensory Disorders at the University of North Carolina. Her research since then is focused on determining the cellular and molecular biological mechanisms by which genetic variations impact human pain perception and risk of development of chronic pain conditions, enabling new approaches to identify new drug targets and treatment responses to analgesics. Dr. Diatchenko has authored or co-authored over 120 peer-reviewed research papers in journals, 10 book chapters, and edited a book in human pain genetics. She is a member and an active officer of several national and international scientific societies.

Dr. Philippe Campeau is a medical geneticist at the CHU Sainte-Justine and the Shriners Hospitals for Children — Canada, and a researcher at the University of Montreal. He did his genetics specialty training at McGill University and his postdoc at the Baylor College of Medicine in Houston. His clinical and research interests include skeletal dysplasias and neurodevelopmental disorders such as DOORS syndrome.

Dr. Roman Krawetz has been in the Department of Cell Biology & Anatomy and the dept. of Surgery at the University of Calgary since January 1, 2013. Dr. Krawetz is a full member of the McCaig Institute for Bone and Joint Health. His lab focuses on stem cell biology and aims to further understand their role in the onset and pathogenesis of Osteoarthritis (OA) and cartilage regeneration. In 2013 he also was awarded the Grace Glaum Professorship in Arthritis Research and in 2015 he was awarded a Tier II Canada Research Chair In Bone and Joint Stem Cell Biology.

Dr. Julie Fradette (PhD) is a Full Professor at Université Laval, Department of Surgery, Faculty of Medicine. She is a researcher at the Centre LOEX de l'Université Laval, at the research center of the CHU de Québec-Université Laval since 2005. She is a stem cell (epithelial and mesenchymal) and tissue-engineering expert focusing on human adipose-derived stem/stromal cells (ASCs) and their use in regenerative medicine. Her most significant contributions are related to the development of substitutes mimicking specific microenvironments, namely adipose tissue, skin and more recently bone. She is the director of ThéCell, the Québec network for cellular, tissular and gene therapy.

Dr. Sylvie Ricard-Blum is a Professor of Biochemistry at the University of Lyon, where she coordinates the Master programme in Biochemistry and is deputy director of the Interdisciplinary Sciences - Health doctoral school. She works on the structure - interaction - function relationships of the extracellular matrix (ECM) using biochemistry, biophysics, bioinformatics and systems biology. Her team has identified hundreds of interactions between ECM proteins, glycosaminoglycans and ECM bioactive fragments called matricryptins, built numerous interaction networks of several ECM proteins and proteoglycans and created the ECM interaction database MatrixDB. Her current research interests focus on the role of intrinsic disorder, supramolecular assembly and disease-associated mutations in rewiring ECM interactomes. She has been Secretary and President of the French Society for Matrix Biology and she is currently President of the International Society for Matrix Biology. She also serves as editor of the HUPO Proteomics Standard Initiative and co-chair of the HUPO-PSI Molecular Interaction group.

Invited Speakers continued

Dr. Kjetil Ask is an Associate Professor in the Department of Medicine at McMaster University. He is the founder and Director of McMaster's Demystifying Medicine Program and the Molecular Phenotyping and Imaging Core facility at The Research Institute of St Joe's Hamilton. Dr. Ask graduated from the University of Burgundy (France) in 2003 and completed his postdoctoral work at McMaster University (Canada, 2004-2008) under the direction of Drs. Gauldie and Kolb and at the NHLBI (US, 2008-2010) with Drs. Martha Vaughan and Joel Moss. He joined McMaster University in 2011 and his lab investigates the cellular and molecular mechanisms involved in progressive fibrotic disease with a special interest in modulating macrophage behavior to prevent progression and stimulate resolution in fibrotic lung disease.

Dr. Edward Harvey, MDCM, M.Sc. is a professor of surgery at McGill University, where he earned his medical degree in 1989. He has obtained an Honours BSc from Western University in biophysics (1985) and a Masters in Experimental Surgery from McGill in 2002. He had further training at Duke University and the University of Washington.

His research interests in fundamental and clinical aspects of bone healing include implant and fracture optimization, biosensors and evaluation of novel hardware and surgical approaches to expedite repair. He collaborates with basic, clinical and engineering scientists and has ongoing industry collaborations, particularly in the field of micro-electromechanical systems (MEMS) and sensors. He is the Chief Investigator of the Injury Repair and Rehabilitation program at the MUHC-RI with over 100 investigators in his group. He has an extensive history of successful peer review funding including CIHR and NSERC amongst others.

Dr. Harvey has had multiple responsibilities on executive and research committees of the Orthopaedic Trauma Association (OTA), the American Academy of Orthopaedic Surgeons (AAOS), the Orthopaedic Research Society (ORS) and the Canadian Orthopaedic Association (COA). He is, or has been, editor-in-chief of the Canadian Journal of Surgery; editorial board member of the Journal of Orthopaedic Trauma and OTA International; chairman of the Research Committee and of the Annual Basic Science Course of the OTA; member of the Board of Specialty Societies Research Committee- AAOS; chair of the Trauma Section- ORS; Member of Institutional Advisory Board- CIHR, and president of the COA.

Dr. Saija Kontulainen PhD, is a Professor in the College of Kinesiology, University of Saskatchewan, Canada. She is also a Fellow of the American Society of Bone and Mineral Research. Dr. Kontulainen completed her PhD in exercise physiology and biomechanics at the University of Jyväskylä, Finland and postdoctoral fellowship in the Department of Orthopaedics at UBC, Canada. She has established a collaborative and interdisciplinary research program focused on bone fragility and fracture prevention across the lifespan. Her bone imaging lab has validated clinical imaging methods to reliably quantify bone (micro and macro) structure and strength estimates, and applied these methods to patient-oriented investigations, including pediatric bone studies and exercise interventions in individuals at risk of fragility fractures.

Dr. David Holdsworth is the Scientific Director of the Bone and Joint Institute and a Scientist in the Imaging group at the Robarts Research Institute. He is also a Professor in the Departments of Surgery and Medical Biophysics in the Schulich School of Medicine and Dentistry, at Western University. Dr. Holdsworth's research program focuses on the development of three-dimensional x-ray imaging systems, for use in diagnosis and therapy. With a team of collaborators, he has developed new methods for musculoskeletal disease detection and treatment for both basic pre-clinical and clinical applications, with an emphasis on dynamic and quantitative imaging of the musculoskeletal system using radiography, CT, and MRI. Most recently, he has expanded his program to include image-based additive manufacturing, which can be used to fabricate patient-specific orthopaedic components.

Dr. Lidan You is an Associate Professor at the University of Toronto. She obtained her PhD degree from City University of New York with Dr. Sheldon Weinbaum on bone fluid mechanics. She did her postdoctoral study at Stanford University with Dr. Christopher Jacobs on bone cell mechanotransduction. Her research focuses on solving biomechanical questions in muscular skeletal system at the cellular level. Specifically, her team works on the anti-resorptive effect of mechanical loading on bone tissue; mechanical loading effect on bone metastasis; vibration effect on bone cell function, and the advanced microfluidic system for bone cell mechanotransduction study.

Poster Session Thursday

Connective Tissues in Development and Disease

T1. **C. Julien**, C. Dsouza, B.M. Willie. Circadian clock regulation of molecular mechanisms underlying load-induced bone formation.

T2. S. Crooks, **S. Condon**, E. Ameri, M. Morris, S.V. Komarova. Systematic review of the effects of space travel on bone health in animals.

T3. **J.L. Ramirez-Garcialuna**, M.A. Martinez-Jimenez, S.A. Abud-Flores, E.S. Kolosovas-Machuca. Digital infrared thermography reduces length of stay for burned patients.

T4. **M. Mannarino**, L. Haglund. Targeting receptors for curcumin and vanillin in painful degenerating intervertebral discs.

T5. **F. Li**, Y. Xie, X. Li, W.W. Du, J. Xu, S. Wang, F.M. Awan, C. Zhou, K. Zeng, B.B. Yang. The role of circ-Itga9 in cardiac remodeling.

T6. **G. Sadvakassova**, K.Steer, K. Tiedemann, N. Mikolajewicz, M. Stavnichuk, Z. Sabirova, I. I.-K. Lee, S. Komarova. L-plastin and PRDX2 are osteoclastogenic factors secreted by actively proliferating erythropoietic cells.

T7. **J.S. Rockel**, B. Wu, S. Nakamura, E. Rossomacha, M. Kapoor. Retro-Inverso Tat-Beclin-1 induces synovial fibrosis and does not protect cartilage from degeneration in a mouse model of OA.

T8. **G. Tavallaee**, C. Sarda, S.A. Ali, E. Rossomacha, K. Shestopaloff, K. Perry, G.M. Mitchell, R. Gandhi, J. Rockel, M. Kapoor. MicroRNA 27b-3p: Role in extracellular matrix regulation in osteoarthritis synovial fibroblasts.

T9. **C. Anderson**, R. St-Arnaud. The FIAT transcriptional repressor as a drug target for bone regeneration.

T10. **Y. Shweiki Alrefai**, M. Naghibosadat, A. McClennan, L. Luyt, L. Hoffman, S. Dhanvantari. Ghrelin and des-acyl ghrelin binding in cardiac tissue are altered with cardiovascular inflammation in Duchenne muscular dystrophy.

Mechanisms of Matrix Remodeling and Disease

T11. **W.W. Du**, L. Fang, W. Yang, F. Li, N. Wu, B.B. Yan. A circular RNA promotes tumor progression.

T12. **O. Mekhael**, J. Imani, M. Padwal, H. Patel, E. Ayaub, A. Ayoub, M. Vierhout, S. Naiel, A. Rullo, J. Hirota, N. Hambly, D. Bridgewater, A. Naqvi, M. Kolb, and K. Ask. ATF6α/CHOP arm deficiency drives lung fibrosis via myeloid-derived macrophages.

T13. M. Rummler, F. Ziouti, **A. Bouchard**, M.E. Lynch, F. Jundt, B.M. Willie. Time-lapse microCT-based in vivo imaging reveals increased bone formation in mice with multiple myeloma bone disease.

T14. **H. Kumra**, V. Nelea, D.P. Reinhardt. Fibulin-4 exerts a dual role in LTBP-4 mediated matrix assembly and function.

T15. H. Sun, **M.T. Kaartinen** Transglutaminase activity and enzyme expression is regulated by M-CSF and RANKL in osteoclasts.

T16. R. Racine, L. Haglund. The effect of pH on disc cells.

T17. N. Dinesh, C.S. Lee, H. Fu, P. Campeau, D.P. Reinhardt. How do fibronectin mutations cause “corner fracture” type spondylometaphyseal dysplasia?

T18. G. Cloutier, T. Khalfaoui, J.F. Beaulieu. Further characterization of the 67 kDa laminin receptor (67LR) in colorectal cancer cells.

T19. M.L. Muthu, K. Tiedemann, T. McKee, V. Nelea, S. Komarova, D.P. Reinhardt. Regulation of adipogenesis and adipose tissue homeostasis by fibrillin-1.

T20. B.D. Quan, M. Wojtas, E.D. Sone. Isomerism affects polyaspartate inhibition of calcium phosphate crystallization.

T21. D. Son, E. Godbout, S. Hume, S. Boo, V. Sarrazy, S. Clément, A. Kapus, B. Wehrle-Haller, L. Bruckner-Tuderman, C. Has, B. Hinz. Kindlin-2 mediates mechanical activation of cardiac myofibroblasts.

Tissue Engineering/Repair/Regeneration

T22. M. Cooke, P. Bayat Sarmadi, D. Rosenzweig. Highly porous elastomer scaffolds for ligament and cartilage tissue engineering.

T23. E. Wong, C.A. Simmons. Design of a biaxial mechanobioreactor for engineering mechanically anisotropic connective tissue sheets.

T24. A. Nour, M. Weber, D. Rosenzweig. Development of a 3D microenvironment model for human bone metastases.

T25. L. Li, R. Fairag, D.H. Rosenzweig, L.A. Haglund. Bone matrix formation by human mesenchymal stem cells on three-dimensional-printed polymer scaffolds.

T26. J. Luo, A. Diaz, B. Gan, R. Grewal, N. Suh, D. O’Gorman. Characterizing the roles of Wilms’ tumor 1 variants in Dupuytren’s disease development.

T27. B. Wu, S. Nakamura, D. Lagares, E. Rossomacha, A. Nakamura, P. Datta, R. Asrafuzaman, P. Monnier, J. Wu, B. Hinz, M. Kapoor. Role of endothelial ephrin-B2 signaling in pulmonary fibrosis.

T28. N. Henley, C. Ngov, N. Boufaied, D. Feng, V. Pichette, C. Gerarduzzi. Integration Analysis of Matricellular Protein Expression Signatures in Mechanistically Different Mouse Models of Kidney Injury.

T29. A.M. Pena Diaz, C. Lau, D.B. O’Gorman. Myofibroblasts derived from palmar fascia fibrosis elicit changes in cytokine gene expression inTHP-1 monocytes.

Poster Session Friday

Connective Tissues in Development and Disease

F1. **K. Tiedemann**, S.V Komarova. Differential regulation of osteoclast fusion and growth.

F2. **A. Ayoub**, M. Padwal, O. Mekhael, M. Vierhout, S. Naiel, S. Abed, A. Dvorkin-Gheva, A. Naqvi, J. Hirota, N. Hambly, M. Kolb, K. Ask. The expression of CCL18 as a unique marker of M2 macrophage in fibrotic lung diseases.

F3. **J.T. Tauer**, S.V. Komarova. Osteocalcin knock-out restores glucose metabolism to wild type levels in a mouse model of osteogenesis imperfecta.

F4. **V. Maranda**, M.H. Gaumond, P. Moffatt. Exploring the pathophysiological cell signalling pathways induced by the osteoblast protein BRIL.

F5. **P. Murphy**, A. McClennan, L. Hoffman. Characterization of Wilms' tumour 1 (WT1) as a fibrotic biomarker for Duchenne muscular dystrophy.

F6. R.A. Zirngibl, A. Wang, Y. Yao, **M.F. Manolson**, J. Krueger, L. Dupuis, R. Mendoza-Londono, I. Voronov. Novel c.G630A TCIRG1 mutation causes aberrant splicing resulting in an unusually mild form of autosomal recessive osteopetrosis.

F7. **W. Xie**, L. Vi, K. Ahmed, T. To, S. Kelley, H. Ullah, P. Kannu. Murine mouse model for Osteofibrous Dysplasia.

F8. **ML. Garcia-Hernandez**, J. Rangel-Moreno, A. Paine, BD. Korman BD, N. Huertas, CT Ritchlin. Dendritic Cell-Specific Transmembrane protein (DC-STAMP) deficiency ameliorates inflammation and joint damage in TNF-driven experimental arthritis.

F9. **D. Taqi**, T. Schwinghamer, J.M. Retrouvey, F. Rauch, F.Tamimi. The prevalence and distribution of missing and unerupted teeth in 171 patients with osteogenesis imperfecta.

Mechanisms of Matrix Remodeling and Disease

F10. **M. Arora**, K.H. Pietiläinen, M.T. Kaartinen. Expression levels of F13A1 transglutaminase in adipose tissue significantly associates with metabolic health in obesity.

F11. **N. Kashyap**, N. Tasevski, M. Ahmed, S. Hakim, Y. Shweiki, B. Wong, A. McClennan, L. Hoffman. Investigating CCN family proteins as potential therapeutic target in Duchenne muscular dystrophy.

F12. **M. Padwal**, O. Mekhael, H. Walker, M. Vierhout, S. Naiel, H. Patel, P. Parthasarathy, J. Imani, E. Ayaub, A.Dvorkin-Gheva, A. Naqvi, J. A. Hirota, N. Hambly, M.R. Kolb, K. Ask. Examining the role of Dectin-1/Clec-7a as a marker of alternatively activated macrophages in idiopathic lung fibrosis.

F13. **B. Qorri**, R.V. Kalaydina, A. Velickovic, Y. Kaplya, A. Decarlo, M.R. Szewczuk. Role of matrix metalloproteinase-9 in extracellular matrix remodeling in disease.

F14. **L. Ho**, A. Yip, F. Lao, F. Botelho, C.D. Richards. Roles of RELMα/FIZZ1 in OSM-mediated lung inflammation and ECM accumulation.

F15. **D. Feng**, N. Henley, V. Pichette, C. Gerarduzzi. The role of SMOC2 in renal cell carcinoma.

F16. **H. Hakami**, C.S. Lee, J. Djokic, A. Pagliuzza, D.P. Reinhardt. Fibulin-4 and latent transforming growth factor-β binding protein-4 cell interactions in elastogenesis.

F17. **S. Khanjani**, M. Blati, A. Philip. CD109 differentially regulates ALK5 versus ALK1 signaling pathways and decreases collagen type II levels and proteoglycan content in articular cartilage in vivo in mice.

F18. **V. Nelea**, S. Wanga, C.S. Lee, H. Hakami, V. Moulin, D.P. Reinhardt. Microfibrillar-associated protein 4 (MFAP4) characterization and functional relevance in the elastic tissues.

F19. **M. Vierhout**, A. Ayoub, S. Abed, S.D. Revill, V. Tat, O. Mekhael, M. Padwal, S. Naiel, A. Hayat, A. Dvorkin-Gheva, A. Naqvi, N. Hambly, J.A. Hirota, M. Kolb, K. Ask. Characterizing the role of IRE1/XBP1 pathway activation in alternatively activated macrophages through molecular phenotyping in interstitial fibrotic lung disease.

F20. **A. Parashar**, J. Marulanda, X. Bai, A.C. Karaplis, M. Cerruti, M. Murshed. Gla residues in MGP protect from phosphate-induced vascular calcification.

F21. **A. Paine**, K. Tiedemann, M. De La Luz Garcia-Hernandez, S. Komarova, C. Ritchlin. Role of DC-STAMP-mediated signaling in cell fusion and osteoclast maturation.

Tissue Engineering/Repair/Regeneration

F22. **A. DeCarlo**, M. Sambi, C. Malardier-Jugroot, M. Szewczuk. Dual mechanism of smart delivery biomaterials.

F23. M. Aziz, **M. E. Cooke**, P. Ahangar, M.H. Weber, D.H. Rosenzweig. Nanoparticle-functionalized poly-methyl methacrylate bone cement for sustained chemotherapeutic delivery.

F24. **T. Huang**, A.M. Pena Diaz,2, K.J. Faber, G.S. Athwal, D.S. Drosdowech, D.B. O'Gorman. Development of a 3D tissue mimetic of shoulder joint infections with Cutibacterium acnes.

F25. **B. Sabano**, M.P. Grant, L.M. Epure, J. Antoniou, F. Mwale. The effect of sLink N on hypertrophic chondrogenesis of BM-MSCs.

F26. **P. Ahangar**, E. Akoury, A. Nour, M.E. Cooke, M.H. Weber, D.H. Rosenzweig. Localized doxorubicin delivery using 3D printed porous scaffolds inside a bioprinted bone-like invitro 3D model.

F27. **R. Fournier**, R.E. Harrison. A method for studying MLO-Y4 osteocyte response to simulated microgravity in embedded 3D collagen droplet scaffolds.

F28. **Y. Lin**, L.M. Hoffman. Characterization of microvasculature in Duchenne muscular dystrophy.

F29. **M. Lodyga**, H. Karvonen, E. Ayab, K. Ask, B. Hinz.v A new mechanism of latent TGF-beta1 presentation by lung macrophages.

ABSTRACTS FOR ORAL PRESENTATIONS

Session I: Connective Tissue Development in Health and Disease

24,25-dihydroxyvitamin D and fracture repair: from mechanisms to clinical trial

Keynote speaker: Dr. René St-Arnaud

Shriners Hospitals for Children – Canada

We have reported that the vitamin D metabolite, 24,25-dihydroxyvitamin D_3 [$24,25(OH)_2D_3$], is beneficial for optimal osteotomy healing in preclinical models (J Clin Invest 128: 3546; 2018). Our results revealed that $24,25(OH)_2D_3$ allosterically activates the transmembrane enzyme FAM57B2 which catalyzes the formation of lactosylceramide (LacCer) involved in chondrocyte differentiation and fracture healing. Mice lacking the $24,25(OH)_2D_3$ synthesizing enzyme, CYP24A1, or the FAM57B2 effector molecule exhibit impaired fracture healing. This phenotype can be rescued by administration of either $24,25(OH)_2D_3$ or LacCer in CYP24A1-deficient animals, but only LacCer could rescue the impaired healing in animals where FAM57B2 was deleted from chondrocytes. This demonstrates that CYP24A1 and FAM57B2 form part of a common genetic pathway to optimize bone fracture repair. Treatment with $24,25(OH)_2D_3$ also reduced healing time and improved biomechanical properties of repaired bones in wild-type animals, suggesting novel approaches to ameliorate fracture healing. We will translate these findings to clinical practice in bisphosphonate-treated osteogenesis imperfecta patients requiring intramedullary rodding surgery with osteotomy to correct deformity of the tibia. In this population of patients, the proportion of delayed union healing is much higher than in the general population, so clinical safety and efficacy could be demonstrated within a cohort of a manageable size.

Involvement of microRNAs in aortic aneurysm formation in Marfan syndrome

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INTRODUCTION: Thoracic aortic aneurysm (TAA) is the leading cause of mortality in Marfan syndrome (MFS), which is characterized by elastic laminae fragmentation, localized inflammatory cell infiltration, loss of smooth muscle contractility, elevated canonical and non-canonical TGFβ signaling. Several microRNAs (miRNAs) are reported to participate in various vascular pathologies, including aneurysm, atherosclerosis, and vascular calcification. Among them, miR-29b inhibition was shown to ameliorate TAA in a MFS mouse model, by regulating ECM synthesis/deposition and rescuing smooth muscle cell (SMC) apoptosis. However, the role of miRNA networks during the TAA formation in MFS is largely unknown. This study investigates the global miRNA expression profiles during the course of TAA formation, and explores the relationship between relevant miRNAs and mRNAs.

RESULTS: To investigate potentially dysregulated miRNAs during TAA formation, microarrays were performed with RNA isolated from the ascending aortae of 4- and 10-week old Fbn1mgR/mgR mice, a fibrillin-1 haplo-insufficient mouse model for MFS. These mice are characterized by TAA starting at about 8-week of age. At 4 weeks, 14 miRNAs were upregulated and 3 miRNAs were downregulated more than 2-fold, comparing the MFS mice with wild-type mice. At 10 weeks after the TAA formed, there were 124 miRNAs upregulated and 5 downregulated, 8 of which were upregulated at both time points. Bioinformatic prediction of the 8 shared miRNAs revealed MAPK, ECM receptor interaction, focal adhesion, mTOR and inflammatory receptor signaling pathways to be dysregulated, all closely related to TAA formation. Comparative mRNA microarrays at 10-week showed 471 upregulated and 253 downregulated mRNAs, 109 (15%) of which reversely correlated with 89 (69%) miRNAs predicted to target them. Gene ontology analysis of the 109 mRNAs revealed the inflammatory response, cell adhesion, MAPK cascade, protein metabolism to be the top hits. miR-122, the most downregulated miRNA at 10-week (-5.87 fold), is predicted to target MCP1 (4.05 fold), CXCL13 (8.74 fold), IL6 (3.03 fold) and IL1b (2.92 fold), which are known to promote the inflammatory response in tissue. Immunohistochemical staining of the mutant aorta displayed infiltration of T-cells and macrophages into the media from the adventitia. miR-221/222, upregulated at both 4-week (2.26/7.3 fold) and 10-week (4.64/3.54 fold), are reported to be necessary for SMC proliferation. This correlates with the downregulation of smooth muscle contractility related genes in the 10-week Fbn1mgR/mgR aorta, indicating a SMC switch to the synthetic phenotype. Additionally, functional analysis showed that the mimics of an upregulated miRNA, let-7g (2.37 fold) can significantly down-regulate total ERK1/2 in SMCs isolated from mouse ascending aortae.

CONCLUSION: 17 miRNAs and 129miRNAs were dysregulated in the ascending aortae of Fbn1mgR/mgR mice at 4-week and 10-week respectively, 8 of which were upregulated at both time points. These miRNAs might contribute to TAA formation by regulating MAPK, ECM receptor interaction, focal adhesion, mTOR and inflammatory receptor signaling. Furthermore, our results suggest the involvement of miR-122 in the inflammatory cell infiltration at the aneurysm site, and miR-221/222's role in the SMC phenotype switch in the course of TAA formation. In addition, the regulatory role of let-7g in ERK1/2 signaling is also highlighted.

The actin binding protein adseverin Is involved in regulating articular chondrocyte phenotype

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INTRODUCTION: Tissue engineering-based therapies for cartilage repair, such as autologous chondrocyte implantation (ACI), requires a large number of cells. Traditional in vitro monolayer culture allows for expansion of articular chondrocyte cell number but also results in phenotypic dedifferentiation which can be characterized by a variety of changes. This is associated with the development of an elongated shape, increased actin polymerization status, development of actin stress fibers, expression of contractile molecules, and downregulation of chondrogenic genes1. Given the changes in actin status during dedifferentiation, the hypothesis of this study is that adseverin, an actin severing and capping protein, plays a role in regulating chondrocyte phenotype and function. The objective is to characterize the role of adseverin in primary articular chondrocytes and passaged/dedifferentiated chondrocytes.

METHODS: Bovine full-thickness articular cartilage was harvested and processed by sequential digestion to isolate primary cells. These cells were transfected as primary cells (P0) with either adseverin siRNA or scrambled siRNA. Alternatively, P0 cells were maintained in culture for passaging twice (P2) and then transfected with either GFP-adseverin plasmid or the empty-GFP plasmid. qPCR and western analysis were used to evaluate gene and protein expression respectively. Cell morphological parameters was acquired through confocal z-stack imaging of the entire cellular profile of calcein AM stained cells and measured using Volocity v6.3 and ImageJ. G- and F-actin protein portions were separated by differential triton solubility and subjected to western blot analysis to quantify G-/F-actin ratio. Data were expressed as mean ± SEM and analyzed using Student's T-test between two groups. One-way ANOVA and Tukey's post hoc was used between multiple groups. Significance was assigned at $p<0.05$. All statistical analyses were performed using GraphPad Prism 6.0 software. Experiments (N) were repeated at least 3 times using independent cell isolations.

RESULTS: Serial passaging of articular chondrocytes in monolayer culture resulted in loss of adseverin protein expression as early as day 14 of culture and remained repressed in P2 cells. Knockdown of adseverin by siRNA in primary chondrocytes promoted an increase in cell size and a spread and elongated shape, actin stress fibers, and decreased G-/F-actin ratio relative to control. The cells also showed increased expression of the contractile genes and proteins, vinculin and α-SMA. These are all features of dedifferentiation. These effects were due to adseverin as its overexpression following transfection of the GFP-adseverin plasmid in P2 chondrocytes reversed all of these changes in part. Furthermore the chondrogenic genes, sox9 and aggrecan were upregulated and collagen type I gene expression was downregulated with adseverin overexpression. The change in aggrecan mRNA expression had functional consequence as these cells exhibited increased total proteoglycan synthesis.

CONCLUSION: These studies suggests that adseverin plays an important role in regulating re-differentiation of passaged chondrocytes and perhaps contributing to maintaining phenotype in primary articular chondrocytes by regulating actin cytoskeleton polymerization status. This will allow us to identify the conditions to generate cells that are suitable to use for cartilage repair.

REFERENCES: 1. Parreno et al., Journal of Anatomy, 2017.

ACKNOWLEDGEMENTS: This work was supported by NSERC Discovery grant and QEII-GSST to BC.

Examining tissue composition, whole-bone morphology and mechanical behavior in Marfan Syndrome

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INTRODUCTION: Marfan syndrome (MFS) is a connective tissue disorder caused by mutations in the fibrillin-1 gene. MFS patients exhibit a range of skeletal symptoms including long bone overgrowth and significantly lower bone mineral density compared to height-matched controls. Expression of fibrillin-1 has been demonstrated in the bone tissue, and a range of cellular effects of fibrillin-1 have been described. However it remains unknown if skeletal symptoms of MFS are caused by the alteration of structural function of fibrillin-1 as a matrix protein, or distortion of its interactions with bone cells. To assess structural effects of the fibrillin-1 mutation, we characterized the bone composition, tissue-level strains and tibia mechanical properties in the Fbn1C1039G/+ mouse model of MFS.

METHODS: The tibiae of 10, 26 and 52 week-old female Fbn1C1039G/+ (MFS) and litter-mate control (LC) mice were analyzed. In vivo strain measurements were performed on the medial-lateral surface of the tibial midshaft to determine the relationship between applied tibial compressive loads and bone tissue deformation. Tibiae were extracted and micro computed tomography (μCT) was used to characterize bone morphology (mid-diaphysis, metaphysis) and curvature at the mid diaphysis. Bone composition was measured in 26-week-old mice with Fourier transform infrared imaging. T-tests were performed to test for significance between Fbn1C1039G/+ and LC mice at $\alpha = 0.05$.

RESULTS: Fbn1C1039G/+ mice exhibited long bone overgrowth and osteopenia consistent with the MFS phenotype. While trabecular bone morphology was similar in MFS and LC mice, tibial metaphysis cortical area and thickness as well as tibial diaphysis cortical area were significantly lower in MFS than in LC. The curvature of the bones measured at the mid-diaphysis was significantly lower in the medial-lateral direction in the MFS mice compared to LC. In terms of composition, the crystallinity was 4% lower in MFS mice compared to LC, implying that the LC mice have mineral with a greater crystal size and perfection than MFS. In vivo tibial stiffness assessed by strain gauging was similar in MFS and LC mice.

CONCLUSIONS: The Fbn1C1039G/+ mice displayed deficits in cortical bone structure, and lower crystallinity in bone mineral, which may be compensated with less curvature in the medial-lateral direction and long bone overgrowth to create structures with a similar stiffness. Finite element analysis is ongoing to further elucidate this relationship. These data provide valuable insights into how changes in the bone matrix affect bone composition, morphology and strength, potentially contributing to the skeletal phenotype in patients with MFS.

AKT pathway in bladder obstruction and matrix-induced smooth muscle phenotype

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Bladder obstruction leads to ongoing changes in smooth muscle cell (SMC) mass, tissue phenotypic changes, and matrix remodeling. However, the underlying mechanisms regulating the obstruction matrix and SMC phenotype are unknown. We hypothesize that an abnormal ECM activates specific molecular pathways in a feedback loop that perpetuates SMC phenopathology. We performed RNAseq on 6 week obstructed bladders in mice. Bladder function was tested using metabolic cages connected to analytical weigh scales and Logger Pro software. Histopathology was assessed by picrosirius red (with optical polarimetry) and H&E staining. Resulting pathways were assessed for activation state, expression, (by Western blotting) and DNA methylation of specific genes in the pathway (with a methylation array), using an in vitro model of human SMC cultured on heat-denatured collagen type I: damaged collagen / DNC) vs. native collagen (NC). RNAseq revealed differential expression of ECM-integrin signaling and PI3K/AKT pathways on DAVID 6.8, including upregulated MMP2, FN1, Col4a1, Col17a1, Igtbl1 and IGF1r. Many of these genes showed significant correlations with functional changes, but Pearson's correlations. DNA methylation analysis by 450K array on DNC vs. NC revealed differential (CpGme) methylation in 12 loci, including decreased CpGme of AKT3. Interestingly, AKT signaling and expression was increased on DNC vs NC by western, concordant with its hypomethylation. This increase was coordinate with increased proliferation and loss of differentiation markers (calponin, SMA, myosin). In addition, inhibition by rapamycin, which alters signaling downstream of AKT, phenorescued SMC differentiation in vitro (myosin expression), and improved bladder physiologic function and pathology during in vivo rat obstruction (1mg/kg/day rapamycin). This work supports a role for extracellular matrix in the epigenetic alteration of AKT expression and differentiation in bladder smooth muscle in vitro, and in obstructive bladder disease in vivo.

Regulation of FGF23 processing and bone mass accrual by the proprotein convertase furin

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Fibroblast growth factor 23 (FGF23) is a hormone secreted by terminally differentiated osteoblasts which regulates phosphate metabolism. Active FGF23 decreases the expression of the sodium/phosphate cotransporter NaPi2a and NaPi2c resulting in a reduced phosphate reabsorption in the kidney proximal tubule. FGF23 proteolytic cleavage by a proprotein convertase (PC) inhibits its activity. Interestingly mutations that render FGF23 resistant to cleavage leads in humans to autosomal dominant hypophosphatemic rickets (ADHR), a rare phosphate wasting disorder characterized by increased FGF23 serum level, hypophosphatemia and osteomalacia. Additional data in mice and humans suggest that FGF23 expression and cleavage are increased in condition of iron deficiency and by erythropoietin. However, the identity of the specific PC(s) involved in FGF23 cleavage in vivo remains undetermined, although in vitro based evidence suggests the implication of furin and/or PC5. Hence, in the current study we investigate the role of furin and PC5 in the regulation of FGF23 in vivo, and assess their specific functions in phosphate metabolism and bone homeostasis. OCN-Cre transgenic mice, expressing Cre recombinase under the control of the human osteocalcin promoter, were crossed with Furinflox/flox or Pcsk5flox/flox mice to generated mice lacking furin or PC5 specifically in differentiated osteoblasts (i.e., Furinosb-/- and Pcsk5osb-/-). The circulating level of intact FGF23 were significantly increased by 25% in Furinosb-/- mice, but remained unchanged in Pcsk5osb-/- mice. However, this increase in active FGF23 did not alter the expression of FGF23 target genes in the kidney of Furinosb-/- mice. Consequently, these mice maintained normal serum phosphate levels and did not show any sign of osteomalacia. Since iron deficiency is required to induce hypophosphatemic osteomalacia in ADHR mouse model (1), we therefore fed control and Furinosb-/- mice a low iron diet for 12 weeks and assess the impact on FGF23 and phosphate metabolism. FGF23 mRNA expression in bones is increased in both control and Furinosb-/- mice fed the low iron diet. FGF23 plasma measurement revealed that Intact/C-terminal FGF23 ratio was ~100% in Furinosb-/- mice versus 77% in control mice, suggesting that furin is responsible of FGF23 processing in vivo in condition of iron depletion. Erythropoietin (rEPO) injection was shown to induce FGF23 expression in bone and bone marrow of rodents (2). Intraperitoneal injection of rEPO at 300U/Kg induced a ~4-folds increase in C-terminal FGF23 and a reduction in Intact/C-terminal FGF23 ratio in both control and Furinosb-/- mice. These results suggest that osteocytes may not be the cells secreting FGF23 in response to EPO or that FGF23 can be cleaved by another PC in osteocytes. Interestingly, despite the absence of osteomalcia, Furinosb-/- mice on normal diet had a complex bone phenotype characterized by a 2-fold increase in trabecular bone volume combined with a 15% reduction in cortical thickness and in overall bone mineral density. Three point bending tests confirmed reduced bone stiffness in Furinosb-/- mice. Together these results identify furin as a PC responsible of FGF23 cleavage in vivo, but also suggest that furin influences bone density through FGF23-independent mechanisms. (1) Farrow et al. PNAS, 2011. (2) Flamme et al. PLoS One, 2017.

Translational control of skeletal muscle stem cell quiescence and self-renewal

Invited speaker: Colin Crist

Lady Davis Institute

Regeneration of adult tissues depends on somatic stem cells that remain quiescent, yet are primed to enter a differentiation program. The molecular pathways that prevent activation of these cells are not well understood. In mouse skeletal muscle, these features are reconciled by multiple translational control mechanisms mediated by microRNA and RNA binding proteins that ensure primed muscle stem cells (MuSCs) are not activated. We show that a general repression of translation, mediated by the phosphorylation of translation initiation factor eIF2α at serine 51 (P-eIF2α), is required to maintain the quiescent state. Skeletal muscle stem cells unable to phosphorylate eIF2α exit quiescence, activate the myogenic program and differentiate, but do not self-renew. Pharmacological inhibition of eIF2α dephosphorylation permits ex vivo expansion of MuSCs that retain regenerative capacity after engraftment into the mdx mouse model of Duchenne muscular dystrophy. We propose a model whereby P-eIF2α ensures in part the robust translational silencing of accumulating mRNAs that is needed to prevent the activation of muscle stem cells and, on the other hand, the selective translation of specific mRNAs that contribute to the molecular signature of MuSC stemness.

ABSTRACTS FOR ORAL PRESENTATIONS

Session II: Genetics and molecular biology of connective tissue disorders

Understanding chronic pain through genomics and transcriptomics

Invited speaker: Luda Diatchenko

McGill University

The Diatchenko lab investigates the psychological, molecular, cellular, and genetic pathways that mediate both acute and persistent pain. A primary goal of her laboratory is to identify the critical elements of human genetic variability contributing to pain sensitivity and pathological pain states. In this talk Dr. Diatchenko will discuss how genome-wide genetic and transcriptomic data analysis can lead to understanding of molecular pathophysiology of pain states and enable individualized treatments and therapies. The analysis of both human clinical samples and animal pain models will be discussed.

Genetic analysis of familial AIS: Implication of ciliary genes

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AIMS: Adolescent Idiopathic Scoliosis (AIS) is a complex disease with unknown etiology characterized by phenotypic heterogeneity. AIS has an important genetic contribution and more than fifteen genes seem to be associated to AIS. Two different approaches were used to identify new candidate genes for AIS including a population-based approach called GWAS and a family-based approach, linkage analysis and whole exome sequencing (WES). In this work, we used family approach to identify new causative genes.

METHODS: We performed WES of at least two affected people from 37 French-Canadian families. In parallel, the linkage analysis that was performed in 25 British families (Ocaka 2007), identified candidate loci and potential variants (using exome sequencing). Gene variants identified by exome sequencing were analyzed by bio-informatics tools and validated by co-segregation with AIS analysis (Sanger sequencing).

RESULTS: Exome sequencing of 37 AIS families, after bio-informatics analysis, identified ciliary candidate genes. With linkage analysis, the locus 9q31.2-q34.2 was identified as significantly linked to the disease in a 5-generation family. Exome sequencing of this region identified a new candidate gene for AIS, a gene coding a protein responsible for ciliary integrity.

CONCLUSION: Exome sequencing of 37 AIS families and linkage analysis identified ciliary genes as possibly involved in spine development. Molecular consequences of these genes will be validated in vitro in cells derived from AIS patients and in vivo in a zebrafish animal model. Almost all identified AIS genes are susceptibility genes. Identifying causative genes is crucial for elucidating AIS pathogenesis.

Development of AVID200, a novel TGF-beta 1 & 3 inhibitor for the treatment of fibrotic diseases

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AVID200 is a rationally-designed isoform specific ligand trap that neutralizes with pM potency TGF-beta1 and -beta3, the two TGF-beta isoforms whose overexpression is closely associated with fibrotic diseases and cancer. AVID200 has minimal activity on TGF-beta2, which reduces the potential for the cardiac toxicity that has been associated with TGF-beta2 neutralization. AVID200 is therefore positioned to be an effective and well-tolerated therapeutic in a variety of clinical settings.

Scleroderma (SSc) is a rare, severe, and progressively debilitating fibrotic disease, affecting predominately middle-aged women. The 10-year survival rate of SSc patients is approximately 55%. No therapy is currently approved for the treatment of SSc, which affects an estimated 20,000 people in Canada. SSc is characterized by widespread abnormalities including immune dysregulation, dysfunction of the vasculature and widespread fibrosis of the skin and multiple internal organs including lungs. TGF-beta is a cytokine that acts as a central mediator of pathogenesis in fibrotic diseases, including SSc. TGFbeta signaling is elevated in fibrotic diseases, and promotes fibrosis by stimulating the transition of fibroblasts to myofibroblasts and the production and deposition of extracellular matrix (ECM) proteins, as well as inducing other mediators of fibrosis. Moreover, the extent of TGF-beta1 and TGF-beta3 upregulation has been shown to correlate with the severity of SSc. TGF-beta is thus an important therapeutic target in fibrotic diseases including SSc. In light of this, we are examining the therapeutic potential of AVID200 in models of fibrotic diseases.

The anti-fibrotic activity of AVID200 was evaluated in a bleomycin-induced murine model of established fibrosis. As expected, injection of bleomycin caused significant weight loss, and fibrosis of the skin and lung. Treatment with AVID200 reduced weight loss and dermal thickness in a dose response manner. Notably, treatment with AVID200 at 5 mg/kg restored the dermis to epidermis ratio to the normal level observed in healthy mice. AVID200 also caused a significant reduction in SMAD2 phosphorylation, a marker of TGF-beta signaling, thus confirming target engagement at the tissue level. In addition, AVID200 resulted in a marked reduction in lung fibrosis.

Taken together, our results demonstrate that AVID200 is effective at reducing fibrosis in a mouse model and is potentially a broadly active treatment for fibrotic diseases, such as SSc. The investigational new drug (IND) application for AVID200 in this patient population has been cleared by the FDA and a Phase 1 clinical study to evaluate AVID200 as a potential treatment for SSc commenced in early 2019.

Dysregulated microRNA expression in osteoclasts from Paget's disease of bone

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BACKGROUND: Paget's disease of bone (PDB) is the second most frequent metabolic bone disorder and is characterized by bone remodeling that is excessive and disorganized. A key pathological feature of PDB involves osteoclasts (OCs) that are larger, more abundant and hyperactive – leading to excessive bone destruction. MicroRNA (miRNA) are small, non-coding RNA that post-transcriptionally regulate DNA expression. Due to miRNA's epigenetic roles, they have been shown in some instances to be reliable markers for disease or pose as potential pharmaceutical targets. Thus, we hypothesized that miRNA profiles may identify overactive osteoclasts causing the bone destruction in PDB.

RESULTS: Through a deep sequencing study to compare the genome wide expression of OC miRNA in patients with PDB versus healthy controls, followed by qPCR validation (n=4/group), we were able to identify 14 miRNAs that appeared to be dysregulated in PDB in the first analyses. These miRNAs were subsequently tested in an independent cohort of patients with PDB, and age- and sex- matched healthy controls (n=10/group), and their expression was normalized (snU6). From these, we selected 4 miRNAs for an in vitro study using cord blood monocyte derived OCs: miRNA-146a-3p and miRNA-155-5p – seen to be decreased in PDB – as well as miRNA-133a-3p and miRNA-31-3p, that were stable in PDB but have known importance within OCs and have been used as positive controls for in vitro studies. We used antagomirs (anti-miRNA oligonucleotides), and mimics to modulate miRNA expression in transfection studies. Non-relevant miRNAs were used as negative controls. Thus far we have demonstrated in vitro, , that miRNA-133a-3p inhibition through siRNA transfection significantly decreases OC bone resorption. miRNA-133a-3p inhibition has also shown through western blot analysis to results in a significant decrease in p38 phosphorylation ($p<0.05$). We have also demonstrated that miRNA-146a-3p, which is seen to be significantly decreased in PDB, when amplified through transfection with miRNA-146a-3p mimics results in a significant decrease in bone resorption ($p<0.01$)– opposing what is seen in PDB patients.

CONCLUSIONS: We found a decrease in the expression of miRNA-146a-3p and miRNA-155-5p in pagetic OCs. We also have confirmed here that miR-146a-3p is involved in OC bone resorption, as has recently been suggested. Further investigation will take place to determine how miRNA-146a-3p is affecting the OC kinome, and to look into the remaining miRNA in question regarding effects on bone resorption and kinome influence. OC miRNA profile might have an important value to yield significant new insights into the OC phenotype in PDB or in other bone diseases with hyperactive OCs.

Progressive bone gain in G6b-B knock-out mice

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BACKGROUND: Bone and bone marrow do not exist in isolation and disruption of one system affects bone-blood system as a whole. In a recent systematic review, we examined bone health in patients with hematopoietic disorders and demonstrated that an increased hematopoietic cell proliferation, such as observed in patients with hemolytic anemias, was associated with bone loss, while bone marrow hypocellularity, such as in patients with chronic myelofibrosis (CMF), was associated with bone density increase. The mechanism of bone gain in CMF is unclear, but it contributes to patients' morbidity as it is associated with bone pain, and mortality as it may lead to bone marrow failure. Recently, a mouse model with a global knock-out of the G6b-B receptor was shown to develop myelofibrosis secondary to megakaryocyte/platelet differentiation defect. Moreover, a group of patients with primary myelofibrosis was identified to have a loss-of-function mutation in the G6b-B gene. Objective: The objective of this study was to characterize temporal changes in the skeleton of the G6b-B knock out mice.

RESULTS: Age- and sex-related changes were examined in 4-, 8-, 16-, and 32-week-old G6b-B+/+, G6b-B+/-, G6b-B-/- female and male mice. G6b-B+/- mice of both sexes were not different from G6b-B+/+ mice. Spleen, a site of extramedullary hematopoiesis, progressively increased in female, but not male, G6b-B-/- mice compared to corresponding G6b-B+/+ mice, reaching 3.2-fold increase at 32 weeks ($p < 0.01$). Liver, another potential site of extramedullary hematopoiesis, was not affected by the mutation. Microcomputed tomography of femur metaphysis of 32-week-old female G6b-B-/- mice demonstrated a 3.8-fold increase in BV/TV and trabecular number and a 2.8-fold decrease in trabecular separation ($p < 0.001$) compared to G6b-B+/+, while in 32-week-old male G6b-B-/- mice trabecular thickness increased 1.3-fold ($p < 0.01$). Femur diaphysis showed the presence of trabecula in female G6b-B-/- mice, with trabecular BV/TV increasing by 24-fold and trabecular number by 20-fold ($p < 0.001$) compared to G6b-B+/+. Mid-shaft cortical bone area was increased 1.2-fold in both female ($p < 0.01$) and male ($p < 0.1$) G6b-B-/- mice compared to corresponding G6b-B+/+. Gene expression analysis of osteoblast, osteoclast, and osteocyte-specific genes as well as TGF-β1, a protein implicated into the development of fibrosis, in bone-derived samples demonstrated no difference between G6b-B-/- and G6b-B+/+ 32-week-old female and male mice.

CONCLUSION: G6b-B-/- mice demonstrate an age and sex-related bone gain, which was especially strong in female G6b-B-/- mice that also demonstrated severe splenomegaly. These data suggest that the observed bone gain is consequent to a hematopoietic disturbance reported in G6b-B-/- mice, rather than alterations in bone cells. This mouse model will be helpful in understanding the pathophysiology and finding new treatments for myelofibrosis.

Understanding growth plate disorders to better treat them

Invited speaker: Philippe Campeau

Center Hospitalier Universitaire Sainte-Justine

Several skeletal dysplasias affect the growth plates (i.e. those with metaphyseal involvement). Such disorders not only cause a short stature, but also cause scoliosis and spinal cord compression, difficulties with joint mobility and ambulation, and joint pain and destruction. Novel therapies are needed to improve the quality of life of affected individuals, but to introduce new therapies, the mechanism of disease has to be well dissected. Increased FGFR3 signaling at the growth plate has long been known to be the cause of achondroplasia, and recently new therapies have immerged to decrease this signaling (through soluble FGFR3, c-natriuretic peptide derivatives, or meclozine). In other disorders (metaphyseal chondrodysplasia type Schmid caused by COL10A1 mutations, and pseudoachondroplasia caused by COMP mutations), ER stress has more recently be identified to play an important role in the pathophysiology. Thus, in pre-clinical models, drugs that decrease this ER stress are also showing promise (carbamazepine, rapamycin, anti-inflammatory or antioxidant agents). We have identified fibronectin as responsible for a form of spondylometaphyseal dysplasia. We are now using patient cells and mouse models to understand the pathophysiology of this disease, with the hope that this will allow us to better treat affected individuals in the long term.

ABSTRACTS FOR ORAL PRESENTATIONS

Session III: Connective Tissue Repair and Regeneration

The role of cell cycle activation in endogenous cartilage regeneration

Invited speaker: Roman Krawetz

University of Calgary

As early as the 1700's, it was observed that the potential of articular cartilage for intrinsic regeneration was minimal. Even very small cartilage defects cannot re-establish their essential low friction surface and tend to degrade further over time and a consequence of this inadequacy is osteoarthritis (OA), a chronic, degenerative joint disorder. Superior cartilage healing post-injury has been observed in the MRL 'super-healer' mouse and has been linked to a deficiency in the cell cycle regulator, p21. Therefore, we have investigated the cell type(s) (e.g. stem/immune) and/or mechanism(s) involved in endogenous cartilage regeneration in $p21^{-/-}$ mice.

To accomplish this, we lineage traced *Prx1*$^{+}$ (transcription factor specific to mesenchymal cells that give rise to bone, cartilage and fat) MSC/progenitors *in vivo* after cartilage injury in $p21^{-/-}$ and C57BL/6 wild-type mice. We further examined the inflammatory cytokine profile of mice in the presence/absence of p21 and functionally tested the role of the CCL2/CCR2 signaling axis, which was found to be altered in $p21^{-/-}$ mice in response to cartilage injury.

Future studies using other lineage reporter mice in the context of p21 deletion will be necessary to determine the cell(s)/factor(s) responsible for cartilage regeneration in these mice and to determine if targeting the cell cycle has potential as a safe and effective therapeutic strategy for treating cartilage injuries and/or OA.

Feasibility of 3D-printed polylactic acid (PLA) scaffolds in promoting bone-like matrix deposition invitro

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INTRODUCTION: Large bone defects represent a significant challenge for clinicians and surgeons. Tissue engineering for bone regeneration represents an innovative solution for this dilemma and may yield attractive alternate bone substitutes. 3D printing with inexpensive desktop printers show promise in generating high-resolution structures mimicking native tissues using biocompatible, biodegradable and cost-effective FDA approved thermoplastics. This study will demonstrate the appropriate pore size to assess the ability of low-cost 3D printed polylactic acid (PLA) scaffolds in conducting primary human osteoblast adhesion, growth and osteogenic matrix deposition. It will also test the hypothesis that human mesenchymal stem cells (MSCs) will adhere, proliferate, and adopt osteogenic phenotype when seeded within these scaffolds.

METHODS: Micro-porous 3D-printed PLA scaffolds with different pore sizes (500 µm, 750 µm and 1000 µm), were designed and manufactured using Flashforge Creator Pro desktop printer. Scaffold properties, mechanical stiffness and material characteristics were assessed using µCT, unconfined compression and contact angel testing respectively. Scaffolds were seeded with 5x105 primary osteoblasts for 21 Days. Cells activity and proliferation were assessed using DNA quantification and scanning electron microscopy. calcified matrix production was visualized and quantified by Alizarin red staining and western blotting. Furthermore, MSCs were seeded within the appropriate pore size scaffold and osteogenic differentiation was evidenced by gene expression, calcified matrix formation and bone-like matrix deposition.

RESULTS: PLA constructs demonstrated high accuracy and reproducibility of fabrication evidenced by µCT. Additionally, scaffolds showed compressive properties comparable to trabecular bone when strained between 5% and 10%. Material surface testing determined hydrophilic features suggesting good interaction with cells. Cells were growing and covering the surface and between the additive layers of the scaffolds indicating cell adaptation to the 3D environment. (750 µm) pore size showed significant statistical superiority ($p < 0.0001$) in cell population expansion, doubling and calcified matrix deposition and over the other sizes. MSCs demonstrated osteogenic phenotypic characteristics evidenced by the significant expression of osteogenic markers (BSP, ALP, RUNX-2, COL-1 and ON), matrix protein (Osteopontin), Calcium deposition and mineralization.

CONCLUSION: The current study focused on emphasizing the value of inexpensive desktop 3D printers and off-the-shelf materials in keeping pace with the advancement of tissue engineering. Three different pore-sized 3D printed PLA scaffolds have been successfully generated using an inexpensive desktop 3D printer. Out of three pore-sizes, (750 mm) scaffold exhibited highest biocompatibility and bioactivity of primary human osteoblasts. For clinical relevance, human MSCs seeded into (750 mm) size scaffolds showed strong osteogenic differentiation capacity. Therefore, we believe this simple, low-cost approach can be extended to future in vivo studies and potential clinical applications incorporating custom-made, on-site and low-cost 3D printed scaffold in healing bone defects.

Accelerating fracture repair by activating Wnt/β-catenin signaling pathway via Tideglusib release

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Bone regeneration includes a well-orchestrated series of biological events of bone induction and conduction. Among them, the Wnt/β-catenin signaling pathway is critical for bone regeneration. Being involved in several developmental processes, Wnt/β-catenin signaling must be safely targeted. There are currently only few specific biologic agents which are FDA approved and already entered clinical trials for enhancing bone healing. A published work has shown that Tideglusib, a selective and irreversible small molecule non-ATP-competitive glycogen synthase kinase 3-β (GSK-3β) inhibitor currently in trial for Alzheimer's patients, can promote tooth growth and repair cavities. Despite some differences, they are some similarities between bone and tooth formation, and we hypothesize that this new drug could represent a new avenue to stimulate bone healing.

A biodegradable FDA-approved collagen sponge was soaked in GSK-3β inhibitor solution or vehicle only (DMSO) and was implanted in 1 x 2 mm unicortical defects created in femora of 35 adult wild-type male mice. Bone defect repair on control and experimental (GSK-3β inhibitor) groups was assessed after 1 week (n=22), 2 weeks (n=24) and 4 weeks (n=24) with microCT and histological analysis for alkaline phosphatase (ALP, osteoblast activity), tartrate resistant acid phosphatase (TRAP, osteoclasts), and immunohistochemistry to confirm the activation of the Wnt/β-catenin pathway.

Our results showed that Tideglusib significantly enhanced cortical bone bridging (20.6 ±2.3) when compared with the control (12.7 ±1.9; p=0.001). Activity of GSK-3β was effectively downregulated at day 7 and 14 resulting in a higher accumulation of active β-catenin at day 14 in experimental group (2.5±0.3) compared to the control (1.1±0.2; p=0.03). Furthermore, the onset of ALP activity appears earlier in the experimental group (day 14, 1.79±0.28; p=0.03), a level of activity never reached at any end-point by the control defects. At 4 weeks, we observed a significant drop in ALP in the experimental group (0.47±0.05) compared to the control (1.01±0.19; p=0.02) and a decrease in osteoclasts (experimental=1.32±0.36; control=2.23±0.67; p=0.04).

Local downregulation of GSK-3β by tideglusib during bone defect repair resulted in significant increase in amount of new bone formation. The early upregulation of osteoblast activity is one explanation of bone healing augmentation. This is likely the effect of upregulation of β-catenin following pharmaceutical inhibition of GSK-3β since β-catenin activation is known to positively regulate osteoblasts, once committed to the osteoblast lineage. As a GSK-3β inhibitor, Tideglusib demonstrates a different mechanism of action compared with other GSK-3β antagonists as treatment was started immediately upon injury and did not interfere with precursor cells recruitment and commitment. This indicates that tideglusib could be used at the fracture site during the initial intraoperative internal fixation without the need for further surgery. This safe and FDA approved drug could be used in prevention of non-union in patients presenting with high risk for fracture-healing complications.

YAP circular RNA, circYAP, attenuates cardiac fibrosis via binding with tropomyosin-4 and gammaactin

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Cardiac fibrosis, characterizing pathological myocardial remodeling, is a worldwide health problem associated with nearly all etiologies of heart diseases. Upon cardiac fibrosis, the compliance of heart tissue was decreased and the progression of heart failure was accelerated. However, molecular mechanisms underlying cardiac fibrosis remain unclear. Yes-associated protein (YAP) is the key component of Hippo pathway which plays crucial roles in cardiac regeneration. The YAP circRNA, circYAP, is generated from exon 5 and exon 6 of YAP pre-mRNA. In our previous study, we found that circYAP played an essential role in cell proliferation and survival. In the present study, we examined whether circYAP modulated heart remodeling. We analyzed the heart tissue specimens from 90 patients with tetralogy of fallot (TOF) and 25 patients with heart failure and found circYAP levels in these patients' heart were significantly decreased compared to 25 hearts without detectable disease. For further investigation, pressure overload animal model was established by transverse aortic constriction (TAC) in mice hearts. Cardiac fibrosis was induced by pressure overload after 8 week in TAC mice. We found that the circYAP levels were significantly deceased in TAC mouse hearts. Upon circYAP plasmid injection, the circYAP levels were brought back to the similar levels as sham group. Meanwhile, the heart function was improved and cardiac fibrosis associated factors, such as collagen I and III and TGF-b, were attenuated by the circYAP plasmid injection. To further investigate the underlying mechanisms by which circYAP functions in cardiac fibrosis, the potential proteins that could bind to circYAP in the cardiac fibroblasts and cardiomyocytes were identified with mass spectrometry. Our results indicated that circYAP probe could pull down more tropomyosin 4 (TMP4) and gamma-actin (ACTG) in cells with circYAP overexpression than vector control. We further confirmed the binding of circYAP with TPM4 and ACTG in cardiac fibroblasts and cardiomyocytes with RNA immunoprecipitation and RNA pull-down assay. Such bindings led to an increased TPM4 interaction with ACTG, which might stabilize actin filaments and inhibit actin-myosin complex formation. We also detected decreased levels of TPM4 and ACTG pulled down by circYAP probes in the TAC mouse hearts due to the decreased circYAP levels. The binding sites of circYAP with TPM4 and ACTG were identified by a computational approach and confirmed experimentally. Collectively, our study uncovered a novel molecule that could regulate cardiac remodeling during cardiac fibrosis and implicated a new function of circular RNA. Our findings also support the pursuit of circYAP as a potential tool for future intervention of cardiac disease.

Low-dose zoledronate for local delivery to patient-derived spinal bone metastasis secondary to lung cancer

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INTRODUCTION: Lung cancer is the leading cause of death from cancer worldwide. Most patients present with metastatic disease at the time of diagnosis, and up to 65% of them develop bone metastases. Bone metastases are not only the most common cause of cancer-related bone pain, but they also lead to additional complications like pathological fractures and spinal compression. Current treatment options for lung bone metastases include chemotherapeutic regimens, excisional surgery and bisphosphonates (BPs), which reduce bone resorption by inhibiting osteoclastic cell activity. Zoledronate (Zol), a high potency third-generation BP, has been shown not only to reduce pain and skeletal-related events in these patients but also to exert direct anti-tumor activity. However, the high systemic Zol doses cause several complications ranging from flu-like symptoms and anemia to osteonecrosis of the jaw and renal toxicity. To overcome these debilitating side effects, we aimed to evaluate the effects of lower Zol doses on lung cancer and lung-induced bone metastases cells over a longer time period, providing an alternative approach to locally deliver Zol at the tumor site.

MATERIALS & METHODS: Human lung cancer (HCC827) and three lung-induced bone metastasis cells (BML1, BML3 and BML4) were first treated with Zol at 1, 3 and 10 μM for 7 days and then assessed for proliferation, migration, invasion and apoptosis. Next, a local delivery method using 3D-printed nanoporous scaffolds loaded with Zol was tested in vitro over 7 days. Statistical analysis was preformed using ANOVA and Tukey post-hoc tests at a 95% confidence level.

RESULTS: Low-dose Zol treatment significantly decreased cell proliferation (HCC827: 10 μM, 86.6% ± 8.8%, p value < 0.001; 3 μM, 68.1% ± 2.85%, p value < 0.001 and 1 μM, 32.7% ± 7.09%, p value < 0.001; combined BMLs: 10 μM, 79.2% ± 12.4%, p value <0.001; 3 μM, 55.3% ± 27.1%, p value <0.001 and 1 μM, 32.6% ± 26.4%, p value = 0.007), migration (HCC827: 10 μM, 55.6% ± 11.98%, p value <0.001 and 3 μM, 23.5% ± 8.1%, p value = 0.019; combined BMLs: 10 μM, 60.2% ± 9.5%, p value <0.001 and 3 μM, 26.2% ± 6.9%, p value = 0.0012) and invasion (HCC827: 10 μM, 16% ± 10.8%, p value = 0.05; combined BMLs: 10 μM, 18% ± 13.3%, p value < 0.001). Also, preliminary data on HCC827 revealed higher levels of apoptotic activity at 10 μM under the same treatment conditions. Moreover, Zol-loaded 3D-printed nanoprous scaffolds released Zol and significantly inhibited lung and metastatic cell proliferation.

CONCLUSIONS: Our data exploits the potential of using low Zol doses for longer treatment periods. At the same time, 3Dprinted drug-loaded nanoporous scaffolds are a potential clinical therapeutic modality to fill bone defects, block cancer recurrence and decrease systemic side effects.

Myofibroblast reprogramming in hypertrophic scars is mediated by adipocytes via BMPs

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OBJECTIVES: Hypertrophic scars can result from surgery (e.g. mastectomy) or burn wounds depending on the wound depth. Patients are not only affected by cosmetic problems but suffer from pain and contractures. Following autologous fat grafting in plastic surgery, significant improvements in morphology and function of hypertrophic scar tissue have been observed repeatedly[1]. However, the underlying molecular mechanisms are largely unknown. Therefore, this project aims to unravel the interactions between adipose tissue and connective tissue and to identify the cell types involved.

METHODS: Myofibroblasts (TGF-β induced and from hypertrophic scars) were stimulated for 24h with conditioned medium from adipose-derived stem cells (ASC) or adipocytes. Following this, protein expression analysis of alpha smooth muscle actin (α-SMA) was performed. Since nuclear receptor PPARɣ interferes with TGF-β-signaling, the contribution of PPARɣ was tested[2]. Therefore, myofibroblasts were treated with PPARɣ antagonist GW9662 (1 µM) followed by 24h incubation with conditioned medium. In addition, PPARɣ expression in myofibroblasts was examined by sub-fractionation. Aiming to identify the key mediator in conditioned media, secretion of bone morphogenetic protein 4 (BMP-4) was analyzed by ELISA and myofibroblasts were treated with BMP receptor antagonist LDN-159189 (200 µM). Additionally, BMP downstream signaling including SMAD 1/5/9 was analyzed as well as effects on α-SMA expression after 24h stimulation with 20 ng/mL BMP-4.

RESUTLS: After 24 h, exposure to conditioned medium from adipocytes but not ASCs induced a significant downregulation of the myofibroblast marker α-SMA on protein level. Notably, this effect was even more pronounced in fibroblasts derived from hypertrophic scar tissue. When myofibroblasts were pre-treated with GW9662, no downregulation in α-SMA could be induced. Analyses of conditioned media identified BMP-4 as a potential mediator. Similar to GW9662, no changes in α-SMA expression were detectable when myofibroblasts were incubated with LDN-159189. Indeed, adipocyte-conditioned medium activated SMAD 1/5/9 complex in myofibroblasts. Interestingly, BMP-4 had a similar reducing effect on α-SMA expression compared to conditioned medium of adipocytes.

CONCLUSION: Downregulation of α-SMA and ECM proteins may indicate a modification of the myofibroblast differentiation state which could be a possible explanation for the regeneration of hypertrophic scars. In line with this reprogramming, PPARɣ as well as BMPs seem to play an important role. Currently, we investigate how BMP signaling and PPARɣ activation may be linked.

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Engineering natural organ specific 3D models for in vitro and pre-clinical studies

Invited speaker: Julie Fradette

Laval University

Various types of tissues and organs can be recreated in the laboratory using tissue engineering strategies. Human reconstructed substitutes can be grafted to replace damaged tissues but they can also be used in vitro to study many biological processes. Work carried out by my team is recognized for its unique scaffold-free reconstruction technique based on human adipose-derived stem/stromal cells (ASCs). We demonstrated that adipose tissue is an excellent source of ASCs for the reconstruction of human substitutes devoid of exogenous or synthetic biomaterials (self-assembly approach). Reconstructed adipose tissue, trilayer skin and more recently bone have been engineered by inducing specific differentiation of ASCs towards the appropriate lineage while stimulating matrix synthesis and deposition. We also developed strategies to further enhance the functionality of these tissues by including a microvascularization component (in vitro-formed capillary networks od endothelial cells) and by using culture conditions ensuring these substitutes would be safe and functional once implanted in vivo (serum-free or xenogen-free protocols). Finally a brief description will be provided of ongoing preclinical studies evaluating the efficacy of these engineered tissues to promote the healing of skin wounds (diabetic and radiation wounds).

ABSTRACTS FOR ORAL PRESENTATIONS

Session IV: Short communications

Role of Toll-like receptors in degenerating scoliotic facet joints

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Adolescent Idiopathic Scoliosis (AIS) is a progressive 3-dimmensional bending of the spine which affects the intervertebral disc (IVD) and the facet joints. Our facet joint characterization study has revealed the presence of degeneration in scoliotic cartilage through decreased proteoglycan content and elevated secretion of inflammatory cytokines and matrix degrading proteases. Recently, a novel degenerative pathway in IVDs and cartilage involving aberrant activation of TLRs was found. When activated by endogenous danger signals (alarmins), TLRs in IVD and chondrocytes initiate the production of inflammatory cytokines, proteases and neurotrophins leading to tissue degradation and pain. In this study, we investigate the potential role of TLRs activation in facet joints from AIS patients as a cause of early degeneration. TLR presence was assessed by rt-qPCR and immunocytochemistry on cells isolated from AIS and cadaveric non scoliotic cartilage samples with consent. TLR2 receptors were activated in monolayer AIS chondrocytes using a TLR2 agonist (Pam2CSK4, Invivogen). Cartilage explants were isolated from the subchondral bone and cultured in the presence and absence of Pam2CSK4 in chondrocyte media, which was analyzed by ELISA for degenerative marker secretion. After the culture period, the cartilage was cryosectionned and stained with SafraninO – Fast green dyes to reveal proteoglycan content. Gene expression analysis revealed a significant upregulation of TLR2 in AIS chondrocytes compared to controls. Interestingly, TLR mRNA in AIS chondrocytes correlated positively and significantly with degenerative factor (MMP3, MMP13, IL-1b, IL-6 and IL-8) mRNA levels. These correlations were mostly absent in non-scoliotic healthy chondrocytes. In the presence of the TLR2/6 agonist, gene and protein expression analysis showed significantly ($p<0.05$) elevated levels of TLR2 receptor, proteases, inflammatory cytokines and pain-related factors such as MMP3, MMP13, IL-6 and NGF. Furthermore, histological staining of cultured cartilage explants revealed a significant decrease in proteoglycan content after treatment with Pam2CSK4 in AIS cartilage, but not in the non-scoliotic controls. In conclusion, our data show that TLR2 activation lead to a degenerative cycle with the upregulation of the receptor itself and secretion of proteases, inflammatory cytokines and pain-related factors that ultimately trigger proteoglycan loss in the affected cartilage. Our study reveals TLRs as a potential therapeutic target to treat degenerating articular cartilage and subsequent pain.

Attenuation of surgically-induced osteoarthritis (OA) by inhibition of autotaxin

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OBJECTIVE: Osteoarthritis (OA) is a chronic, progressive joint disease leading to a poor quality of life. Previously, we reported that select Lysophoshatidylcholines (lysoPCs) are increased in animal models of OA (Datta et al., 2017, PMID: 28811491). We also identified that these particular metabolites may promote OA pathogenesis in an autotaxin (ATX) – dependent manner. ATX is an enzyme responsible for the conversion of lysoPC to the inflammatory mediator lysophosphatidic acid (LPA). Previous studies have also shown that ATX levels in plasma and synovial fluid correlate with the severity of knee osteoarthritis (Mabey et al., 2015, PMID: 25659292). Thus, we sought to identify if local pharmacological inhibition of ATX can attenuate surgically-induced OA in vivo and its mechanism of action in vitro.

METHODS: 9 week-old mice were subjected to surgically-induced OA. ATX antagonist (PF -8380, Pfizer) was injected intraarticularly in the knee joints at 2nd,4th 6th and 8th week of post-surgery and subsequent knee joint pathology was evaluated. Primary human chondrocytes were treated with IL-1β/ATX antagonist, and the expression of catabolic markers was determined.

RESULTS: Local injection of ATX antagonist reduced the degree of cartilage degeneration in surgically induced OA models compared to saline injected controls. ATX antagonist also reduced the amount of synovitis as compared to controls. Immunohistochemical analysis of mouse knee joints showed decreased expression of catabolic markers (C1-2C and MMP 13) in ATX treated mice compared to control mice. In vitro, ATX antagonist attenuated IL-1β-induced increases in the expression of MMP13.

CONCLUSION: Inhibition of ATX attenuates surgically-induced OA. We are currently investigating the mechanism of action of ATX in attenuating OA. Our data, to date, suggests pre-clinical efficacy of ATX to limit OA progression.

Combination treatment of novel ActRIIB ligand Trap and Zolendronate improves bone-muscle proprieties in osteogenesis imperfecta

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Osteogenesis imperfecta (OI) caused by mutations disturbing the production or processing of the collagen type I protein, characterized by fragile bones and low muscle mass and function. Activin A and myostatin, members of the TGF-b superfamily, are involved in an important role in the control of muscle mass and in muscle-bone communication. We investigated activin A/myostatin signaling in a mouse model of severe dominant OI, Col1a1Jrt/+ mouse (n=8mice/group), and the effect of activin A/myostatin inhibition by a soluble activin receptor IIB trap, ACE-2494 (10mg/kg twice a week), in combination with zolendronate (0.05 mg/kg three times per week), on bones and muscles in 4-week old male mice. Previously our group has shown that compared to wild type mice, Col1a1Jrt/+ mice had elevated TGF-b signaling in bone and muscle tissue. ACE-2494 treatment of Col1a1Jrt/+ mice resulted in 80% increase in muscle mass ($p<0.0001$), bone length was increased 2-4%, but cortical thickness and the mechanical proprieties of the femur were not improved. Therefore, to improve these results we decided to combine this therapy with zolendronate. The combination treatment resulted in the observed gain in muscle mass and significantly improvement in bone length but also in an improvement in cortical thickness of 4% ($p<0.0001$) and bone mass by 200% ($p<0.0001$). Therefore, we can conclude that activin A/myostatin ligand trap ACE-2494 is effective in stimulating muscle mass and bone length diaphyseal and in combination with zolendronate we can improve in addition the bone mass and the cortical thickness phenotype observed in dominant OI.

Age-associated changes in purinergic mechanotransduction

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BACKGROUND: The mechanical environment plays a major role in bone remodeling and exercise is an effective way of improving and maintaining bone health. However, the skeleton's ability to adapt to mechanical loading declines with age. The mechanism(s) responsible for this age-related loss in mechano-responsiveness are unknown. ATP and ADP are among the earliest biochemical signals released by mechanically-stimulated bone cells that act via the purinergic (P2) receptor family to evoke downstream intracellular calcium $[Ca^{2+}]_i$ elevations. Objectives. The aim of this study was to determine whether mechanotransductive purinergic signaling alters with age. We evaluated ATP release and downstream signaling in osteoblasts isolated from young and old mice.

METHODS & RESULTS: Compact bone-derived osteoblasts (CB-OBs) were isolated from 10- and 70-week old C57Bl/6J mice by enzymatic digestion and cultured in osteogenic media for 3 days prior to experiments. We observed no age-related differences in the amount of ATP released following mechanical stimulation of CB-OB cells by turbulent fluid shear applied by displacing 50% media 10 times over cell layer. However, when total extracellular ATP and ADP content in tibial and femoral explants was measured, we found 10-fold more ATP and ADP in bones from young mice, compared to older mice. When individual Fura2-loaded CB-OB cells were mechanically-stimulated by a glass-micropipette, the oscillatory fraction of calcium responses in neighboring non-stimulated cells was significantly higher in CB-OBs isolated from older mice. Consistently, there was a significant age-related difference in the dose-dependence of Ca^{2+} responses to [ATP]. Finally, we demonstrated that mRNA expression of the P2RY12 receptor was significantly elevated in 70-week old CB-OB, compared to younger mice.

CONCLUSION: These data suggest that increasing age is associated with (i) decrease in basal extracellular [ATP] and [ADP], (ii) increase in P2Y12 expression, and (iii) alterations in mechanotransductive purinergic signaling, which together may contribute to lower mechano-adaptive response in the aging skeleton.

Endotypes of primary osteoarthritis identified by a metabolomics approach

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BACKGROUND: Osteoarthritis (OA), the most common form of arthritis, is a heterogeneous disease of overlapping distinct conditions that have different etiologies but similar clinical manifestation, which is the major contributing factor to the failure of clinical trials to detect efficacy of the disease modifying OA drugs. Efforts have been made to classify subtypes of OA patients based on either epidemiological risk factors or structural changes on MRI, but significant overlapping features among the OA subtypes defined by these methods limit their clinical application. We hypothesized that endotypes of OA exist and can be identified by a metabolomics approach and tested our hypothesis in the well-established Newfoundland Osteoarthritis Study (NFOAS).

PATIENTS & METHODS: The study participants were from the NFOAS that comprised total hip or knee replacement patients who were recruited between 2011 and 2016 in St. John's, NL. OA diagnosis was made based on American College of Rheumatology OA criteria and post-surgery pathology reports on cartilage. Plasma samples after at least 8 hours fasting were collected from all patients and metabolomic profiling was performed using the Biocrates AbsoluteIDQ p180 kit. Factor analysis was utilized to reduce the number of metabolites based on their relationship and the identified factors were used in the subsequent analysis. K-means and hierarchical clustering were applied to identify endotypes of OA patients, and logistic regression was used to identify the most significant metabolites that contribute to the classification of the endotypes of OA patients.

RESULTS: A total of 614 patients were included in the study. Mean age was 65±8.7 years and mean BMI 33.71 ±6.9 kg/m2. 58% were females. Among 186 metabolites measured, 162 passed the quality control process and included in the analysis. Factor analysis identified 13 distinct factors. Based on these factors, K-means and hierarchical clustering identified two main subgroups, group A with 132 patients and group B with 482 patients. Three unique metabolites – Lysophosphatidylcholine acyl C26:1, phosphatidylcholine diacyl C40:2, and phosphatidylcholine acyl-alkyl C36:3 were identified as the major contributors for the grouping. These metabolites implicate the alteration of the phosphatidylcholine to lysophosphatidylcholine conversion pathway. Group B was further clustered into four distinct subgroups, B1 with 256 patients, B2 with 62 patients, B3 with 59 patients, and B4 with 105 patients. Arginine and citrulline were the major contributors for B1, lysine, serine, threonine, tyrosine, and methioninesulfoxide biogenic amine were for B2, and acylcarnitine and sphingomyeline related metabolites for B3 and 4.

CONCLUSION: Our data suggest that there are five endotypes existing in primary OA, each characterised by metabolic alteration involved in phosphatidylcholine to lysophosphatidylcholine conversion, arginine metabolism, beta-oxidation pathway, sphingomyeline metabolism, and protein phosphorylation. Our results provide new insights into the pathogenesis of OA and novel targets for developing disease-modifying OA drugs.

ACKNOWLEDGEMENTS: We thank all the study participants who made this study possible and all the staff in the NFOAS who helped the sample collection. Metabolomic profiling was done at The Metabolomics Innovation Center (TMIC). The study was supported by Canadian Institutes of Health Research, The RDC of NL, and Memorial University.

Load-induced bone formation in mice is not affected by time of loading

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Bone adapts to mechanical loading by forming and resorbing bone to meet functional demands. It has previously been shown that bone formation markers oscillate based on time of day. The aim of this project is to determine how the time of day affects the bone formation and resorption response to mechanical loading. In vivo cyclic compressive loading was applied to left tibiae of 10-week-old C57BL/6 female mice for 2 weeks (+1200με at midshaft determined by strain gauging, 216 cycles/day at 4 Hz) and right tibiae served as internal controls. Loading was performed daily (Mon-Fri) at either Zeitgeber (ZT)2 or ZT14. The mice were housed in metabolic cages and in-vivo microcomputed tomography was performed. Results indicate that loading led to significant increases in cortical and trabecular microstructural parameters (e.g. cortical area, cortical thickness, bone volume fraction, trabecular thickness) in the loaded compared to the control limb. No significant differences in bone mass or microstructure were observed after loading between the ZT2 or ZT14 time points. Further studies will analyze longer loading periods and other loading time points to determine if time of loading affects molecular or material level properties. These data may enhance load-included bone formation in patients undergoing exercise.

The effect of cross-linking and net charge on collagen mineralization

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Intrafibrillar collagen mineralization is a crucial process for the formation of healthy bone and dentin. Numerous factors can affect collagen mineralization, including acidic phosphoproteins, proteoglycans, and citrate. Charged macromolecules can stabilize amorphous calcium phosphate particles and direct intrafibrillar mineralization of collagen. Several models of collagen mineralization have been proposed and the importance of charge in the collagen mineralization has been highlighted. Collagen itself may also influence mineralization; it is known that natural collagen cross-linking differs between tissues and change with age. Artificial cross-linking can affect the rate of collagen mineralization, though the exact mechanism has not been revealed in detail. Collagen cross-linking affects mechanical properties and stability of collagen fibrils and also it affects the charge of collagen. However, it is unknown with of those features cause the different rate of collagen mineralization. In this study, we try to understand the interplay between collagen cross-linking and charge in the context of mineralization.

The aim of this study is to examine how cross-linking and other chemical modifications of collagen affect its mineralization, with particular attention to net charge and charge distribution. Collagen fibrils were assembled by raising the pH of acid-solubilized collagen and subsequently were chemically modified to introduce crosslinking and/or to alter the net charge. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide was used to cross-link the collagen fibrils without changing the net charge, while glutaraldehyde fixation was used to cross-link and lower the net charge of collagen fibrils. The net charge of collagen fibrils was modified by neutralizing amino groups of lysine residues by acetylation, while carboxyl groups of aspartate and glutamate residues were neutralized by coupling with glycine methyl ester. The number of cross-links and modified amino groups was quantified with colorimetric assays. Chemically modified and unmodified collagen fibrils were mineralized in vitro using a metastable calcium phosphate solution stabilized by polyaspartic acid, and the rate of mineralization, crystal morphology and orientation was estimated based on transmission electron microscopic examination.

Our results show how collagen cross-linking and altered charge affect mineralization and how collagen might direct intrafibrillar mineral formation.

The regulation of autophagy via YAP signaling in systemic sclerosis

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Systemic Sclerosis (SSc) is an autoimmune disease of unknown etiology characterized by progressive fibrosis of the skin and various internal organs. Activated fibroblasts, also known as myofibroblasts, are the key contributors to fibrosis in Scleroderma patients. The active fibroblasts caused abnormal remodeling and excessive deposition of extracellular matrix (ECM), which resulted in the stiffening, cellular dysfunction of connective tissues and organ failure eventually. Although the signaling pathways underlying fibrosis are not well understood, recent studies have shown yes-associated protein (YAP) is emerging as a key player in the fibrotic process and targeting YAP may have therapeutic effects for SSc. It has been shown that YAP signaling regulates the cellular responses including autophagy, which involves the degradation of redundant or defective cell components. However, the relevance to fibrotic process in SSc remains unclear.

In order to characterize the YAP-dependent regulatory mechanisms underlying the fibrosis of connective tissue, we have probed the expression of YAP in a panel of fibroblast cell lines at the genetic and protein level. Following the induction of autophagy in these cells, we performed the quantitative measurement of YAP transcriptional activity, autophagy responses (LC3B expression), production of fibrillar type I and type III collagens, initiation of expression of α-smooth muscle actin (α-SMA)), a molecular marker of activated myofibroblasts. Then we introduced a YAP inhibitor (peptide 17) to block its interaction with the nucleus target TEAD and then investigated the role of YAP signaling in the autophagy response and fibrosis in SSc.

Our results showed that the expression of YAP is significantly up-regulated in myofibroblasts and the nucleus entry of YAP plays a critical role in autophagy responses. The blocking of YAP-TEAD interaction decreased the production of fibrillar type I, type III collagens, and the expression of α-SMA. Therefore, our results suggested that YAP signaling regulates the autophagy level inside the fibroblast and this regulation might be critical for the fibrotic process in SSc.

Fibulin-4 and latent transforming growth factor-β binding protein-4 in wound healing

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INTRODUCTION: Wound healing is a highly complex process producing only partially functional scar tissue. This tissue lacks the integrity of organized elastic fibers as well as of collagen fibers, which contributes to the stiffness of scar tissue. Fibulin-4 (FBLN4) and latent transforming growth factor beta binding protein-4 (LTBP4) are required for elastic fiber formation. FBLN4 also plays a significant role in collagen fiber assembly. Knockdown of LTBP4 in skin fibroblasts isolated from systemic scleroderma patients prominently reduced downstream collagen type 1A1 and 1A2 mRNA levels. Additionally, mutations in FBLN4 and LTBP4 cause autosomal recessive cutis laxa (ARCL) type B and C, respectively. On the molecular level, it is not known how FBLN4 and LTBP4 function in wound healing.

RESULTS: Here, we analyzed human normal and scar skin samples for their elastic fiber protein profile. FBLN4 and LTBP4 localized specifically to elastic fibers and collagen fibers in normal and scar skin tissues from different ages. To investigate the roles of FBLN4 and LTBP4 in the proliferation phase of wound healing, cell proliferation and cell migration assays were performed. FBLN4, but not LTBP4, elevated skin fibroblast proliferation. Both, FBLN4 and LTBP4 enhanced skin fibroblast migration. To further investigate the role of FBLN4 and LTBP4 in cell migration, focal adhesion and actin filaments were analyzed in skin fibroblast cultures seeded on FBLN4 or LTBP4. Both proteins stimulated focal adhesion kinase phosphorylation through RhoA activation. We utilized collagen gel contraction assays to evaluate the function of FBLN4 and LTBP4 in the contractility of myofibroblasts, a key cell type in wound healing. The presence of FBLN4 and LTBP4 increased significantly the contraction of myofibroblast-cellularized collagen gels.

CONCLUSIONS: Overall, the results suggest that FBLN4 and LTBP4 have important roles in wound healing since they promote cell migration, focal adhesion formation and myofibroblasts contractility.

Characterization of Wilms' tumour 1 (WT1) as a fibrotic biomarker for Duchenne muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is the most commonly inherited pediatric muscle disorder. DMD has no cure, and most patients succumb to the disease in their mid-twenties. It is characterized by muscle degeneration, resulting from the loss of the cytoskeletal protein dystrophin. Poor dystrophin function, or reduced production, leads to a decrease in structural support in muscle cells. These cells become prone to apoptosis, resulting in remodeling of muscle into fibrous and fatty connective tissue, impeding overall muscle function. Fibrosis is profound in DMD, generating a microenvironment that is a significant impediment to both endogenous muscle repair and any potential regenerative strategy. At present, there are few therapies that specifically target fibrosis and microenvironment improvement, but one possibility rests in targeting the Wilms Tumor 1 (WT-1) protein. WT-1 is a zinc finger transcription factor commonly found in nephroblastomas, and recently shown to be expressed in fibrotic conditions such as Dupuytren's disease and pulmonary fibrosis.

The objective of this research project is to characterize WT-1 expression in DMD muscle tissue. We hypothesize that WT-1 is upregulated in DMD muscle tissue prior to onset of fibrosis.

To provide an understanding of WT-1 function in DMD, our methodology makes use of wildtype control mice and mdx DMD model mice. Subtypes of these mdx mice, with one or both alleles for utrophin knocked out, and at young, mature, and aged timepoints, were also used to provide models for different fibrosis forms. We utilized immunohistochemistry to quantify WT-1 protein expression, and Masson's Trichrome staining to quantify fibrosis, in muscle tissue taken from the heart, diaphragm and gastrocnemius of these mice. One-way ANOVA with Bonferroni's post-hoc test was used to identify significant differences between groups.

WT-1 expression was found to be elevated in the diaphragm of mdx mice compared to wildtype controls, but not in the gastrocnemius muscles of these same mice. Furthermore, WT-1 expression was found to be elevated in both diaphragm and gastrocnemius muscles of mdx/utrn+/- and mdx/utrn-/- mice compared to wildtype controls. This effect was found in young and mature mice, but no effect was seen in aged mice. Only the mdx/utrn+/- and mdx/utrn-/- gastrocnemius and diaphragm were found to be fibrotic in mature mice, and these in addition to the mdx diaphragm were found to be fibrotic in aged mice.

Therefore, the hypothesis that WT-1 is upregulated in DMD muscle tissue prior to fibrosis was supported. These results suggest that WT-1 is a broad marker for fibrotic conditions, rather than being limited to conditions such as pulmonary fibrosis. With regards to future work, the impact of WT-1 on the progression of fibrosis still requires investigation. Clinical applications involving WT-1 are numerous, most notably the foremost application that WT-1 may be used as a biomarker to detect the onset of fibrosis. Additionally, immunotherapies currently exist which target WT-1 positive cancer cells for destruction by the immune system. Should WT-1 contribute to fibrosis rather than only acting as a biomarker for it, whether the application of these therapies impedes fibrosis overall can be examined.

ABSTRACTS FOR ORAL PRESENTATIONS

Session V: Fundamentals of extracellular matrix biology

Insights into the structure, interactions and functions of a matricryptin, the pro-peptide of lysyl oxidase

Invited speaker: Sylvie Ricard Blum

University of Lyon

Lysyl oxidase (LOX) catalyzes the first step of the cross-linking of collagens and elastin. LOX is secreted as a proenzyme and is activated by Bone Morphogenetic Protein-1, which releases its catalytic domain (32 kDa) and its N-terminal propeptide. The propeptide (30 kDa) is a bioactive fragment or matricryptin, which inhibits angiogenesis and promotes adipogenesis. We characterized the propeptide by biophysical techniques (circular dichroism, dynamic light scattering, and small-angle X-ray scattering - SAXS) and showed that it is disordered, flexible and elongated (Dmax: 11.7 nm, Rg: 3.7 nm). 3D models of the propeptide were generated by coarse-grained molecular dynamics simulations restrained by SAXS data. The R158Q loss-of-function polymorphism described in breast cancer patients induced the aggregation of the propeptide, which could prevent it to interact with its partners. We have identified 17 new binding partners of the propeptide by label-free assays. They include glycosaminoglycans (hyaluronan, chondroitin, dermatan and heparan sulfate), collagen I, cross-linking and proteolytic enzymes (lysyl oxidase-like 2, transglutaminase-2, matrix metalloproteinase-2), a proteoglycan (fibromodulin), Epidermal Growth Factor (EGF), and a membrane protein (tumor endothelial marker-8). This suggests new roles for the propeptide in ECM assembly and cross-linking, cell-matrix adhesion, and in the regulation of EGF signaling pathway.

Inhibiting fibrotic encapsulation of body implants by targeting mechanical activation of profibrotic TGF-β1

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BACKGROUND: The clinical performance of reconstructive silicone implants, such as breast implants is often compromised by foreign body reactions (FBRs). FBRs culminate in the formation and contraction of fibrotic collagen tissue by α-SMA-expressing myofibroblasts contributing to implant failure. TGF-β1 is a key driver of fibrosis and secreted into the matrix in complex with the latent TGF-β1 peptide (LAP). Cell pulling on LAP via αv integrins mechanically activates TGF-β1 in context of a resistant matrix. Mechanical stress also controls the binding strength of integrins to their matrix ligands. The mechanisms and integrins activating TGF-β1 in FBRs are unknown.

HYPOTHESIS: The stiff surface of silicone implants enhances activation of αvβ1 integrin and - consequently - TGF-β1.

OBJECTIVE: To test whether and how inhibition of TGF-β1-activating integrins prevents biomaterial encapsulation without causing the severe inflammatory side effects reported for global TGF-β1 inhibition in fibrosis.

METHODS: Silicone implants and osmotic pumps releasing the αv integrin-specific inhibitor CWHM-12 were implanted subcutaneously in transgenic mice, expressing GFP and RFP under the control of the Col1α and α-SMA promoters, respectively. Implants were excised, and fibrotic capsules analyzed for thickness, collagen content, myofibroblasts recruitment (RFP/GFP-positive cells), and αv integrin and β1 integrin localization using immunohistochemistry. For in vitro studies, stiff (3,000 kPa) and soft (3 kPa) implant silicones were coated with recombinant LAP and used as substrates for mouse fibroblast cultures. Integrin β1 activation and recruitment to focal adhesions was quantified on soft and stiff substrates and modulated intracellular stress.

RESULTS: Higher accumulation of collagen, myofibroblasts, αv integrin and β1 integrin was found at the surface of implants in control mice. In contrast, CWHM-12 administration significantly reduced fibrotic capsule thickness, collagen deposition, and myofibroblast accumulation around implanted biomaterials 1- and 4-weeks following implantation. In vitro, blocking of αv integrin with CWHM-12 resulted in a decreased adhesion capacity and cytoskeletal force transmission of fibroblasts to LAP, concomitant with reduced mechanical activation of latent TGF-β1 from the matrix. Fibroblasts cultured on stiff LAP-coated implant surfaces displayed augmented activation and recruitment of integrin β1 to focal adhesions compared to cells cultured on soft silicone surfaces. Higher intracellular stress induced by contraction agonists resulted in increased integrin β1 recruitment to focal adhesions.

CONCLUSION: The αv integrin-mediated mechanical activation of TGF-β1 is an important factor driving the encapsulation of biomaterial implants. The affinity of αvβ1-binding to LAP increases with increasing mechanical stress in the fibrotic environment and the contractile activity of the integrin-expressing cells. Interfering in this mechanism with αv-specific RGD peptidomimetics, such as CWHM-12, possibly decreases the fibrotic response to implants, leading to a prolonged lifetime and reduced complication rate.

Cytokines enhance interactions between TßL1, TßLR1, Small Ubiquitin-like MOdifiers (SUMOs) and ß-catenin in palmar fascia fibroblasts

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BACKGROUND: Dupuytren's disease (DD) is a benign fibrosis of the palmar fascia, the major connective tissue layer beneath the skin of the palm and digits. This common, heritable condition can cause permanent palmar-digital contractures and loss of hand function. While contractures can be released by surgery or by non-surgical treatments, these approaches do not prevent disease recurrence and more effective treatments are needed. Many fibroproliferative diseases, including DD, contain fibroblasts with increased cytoplasmic and/or nuclear levels of ßcatenin. Translocation of ß-catenin from the cytoplasm to the nucleus promotes the trans-activation of oncogene expression in cancers, and anti-cancer therapeutics have been developed to attenuate the translocation process. Their mode of action is to competitively inhibit interactions between ß-catenin and the proteins that facilitate its nuclear import, SUMOylated (Small Ubiquitin-like MOdified) Transducin ß-like 1 (TßL1) and Transducin ß-like Receptor 1 (TßLR1). It may be possible to cross-purpose anti-cancer therapeutics that attenuate nuclear translocation of ß-catenin, and the trans-activation of genes that promote fibroproliferation, to treat benign but highly recurrent fibroses such as DD. Chronic inflammation is hypothesized to contribute to the pathogenesis of DD and to many other fibroses. The aims of this project were to determine if SUMO moieties, TßL1, TßLR1 and ß-catenin form complexes in primary fibroblasts derived from patients with DD, and if cytokine treatments designed to mimic chronic inflammation modified their interactions.

METHODSs: Primary fibroblasts were isolated from palmar fascia explants derived from patients with DD (DD cells) or undergoing unrelated hand surgeries (control, CT cells). DD and CT cells were cultured with or without "cytomix" (TGFß1, IL4, IL10, 2ng/ml) treatments for 48 hrs. Immunocytochemistry and Proximity Ligation Assay (PLA) analyses were performed to detect and assess interactions between proteins using antibodies to TßL1 and TßLR1 (mouse), SUMO1 and ß-catenin (rabbit) and complimentary oligonucleotides conjugated to anti-mouse and anti-rabbit secondary antibodies (PLA). Two and three dimensional (Z-stack) images were captured and processed on a Nikon A1R confocal microscope.

RESULTS: Similar levels of SUMOylated TßL1 and TßLR1 were detected in untreated DD and CT cells. TßLR1/ß-catenin interactions were abundant, whereas TßL1/ß-catenin interactions were relatively rare, in these cells. Cytomix treatments significantly increased SUMO moiety/TßL1 interactions in DD cells but not in CT cells, while SUMO moiety/TßLR1 interactions were unaffected by treatment. TßL1/ß-catenin and TßLR1/ß-catenin interactions were significantly increased by cytomix treatments in both DD and CT cells.

DISCUSSION: Overall, our findings are consistent with a ß-catenin nuclear translocation model where SUMOylated TßL1/ßcatenin interactions are the least abundant, potentially "rate limiting", step in forming SUMO-TßL1/SUMO-TßLR1/ß-catenin complexes. The novel finding of cytokine-induced SUMOylation of TßL1 may represent one mechanistic link between inflammation and fibroproliferation in DD. We are currently testing the relative sensitivities of DD and CT cells to a small molecule inhibitor of SUMO-TßL1/SUMO-TßLR1 interactions with ß-catenin ("Tegavivint", Iterion Therapeutics). Elucidating molecular mechanisms that DD and cancers may have in common will help to determine the utility of cross-purposing anticancer drugs such as Tegavivint for the treatment of DD and other benign fibroses.

Elucidating the functional role of miR-34a in osteoarthritis pathogenesis – Involvement in obesity and osteoarthritis

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PURPOSE: We have previously reported that microRNA-34a (miR-34a) is expressed at significantly higher levels in the synovial fluid of end-stage knee osteoarthritis (OA) patients compared to early OA. Studies have shown that miR-34a levels are also elevated in obesity. Despite the strong association between obesity and OA pathogenesis, no studies have examined the role of miR-34a in the development of OA during obesity. We hypothesize that during obesity expression of miR-34a is elevated and contributes to OA pathophysiology.

METHODS: Mouse blood was collected at 9 weeks old (baseline) and at the end of a high-fat diet (HFD) or lean diet (LD) course. Human plasma was obtained from late-stage OA patients undergoing total knee replacement (TKR); cartilage and synovial tissue were obtained during TKR. OA patients with no co-morbidities were segregated according to body mass index (BMI) into non-obese (BMI=18.5-29.9 kg/m2) and obese groups (BMI≥30kg/m2). Chondrocytes and synovial fibroblasts (SF) were transfected with 100nM miR-34a mimic or antisense oligonucleotide (ASO). For in-vivo studies, 9 week-old mice were injected once with a 5ug of in-vivo grade mir-34a mimic or scrambled oligonucleotide (SCO) and joints were collected 8 weeks post-injection for histology. 9 week-old mice were subjected to destabilization of the medial meniscus (DMM) to induce OA and injected 2, 4, and 6 weeks post-surgery with a miR-34a ASO (5ug) or SCO. Joints were collected 10 weeks post-surgery. Heterozygous miR-34a knock out (KO) mice were bred to generate global homozygous KOs and mouse articular cartilage was isolated to culture chondrocytes.

RESULTS: TKR patients expressed significantly increased miR-34a in plasma, cartilage, and synovial tissue compared to healthy controls/early OA patients. In mice, miR-34a was sig. overexpressed in knee joints (cartilage and synovium) at 10 weeks post-DMM compared to sham. To identify the biological effects of miR-34a on chondrocyte and SF gene expression, functional studies were conducted in vitro. Chondrocytes treated with miR-34a mimic had a sig. reduction of SIRT1 (a direct target of miR-34a), anabolic and autophagy markers, as well as, elevated catabolic markers. Chondrocytes treated with miR-34a inhibitor reversed these effects. SFs treated with miR-34a mimic expressed sig. elevated inflammatory, fibrotic, and autophagy markers. SFs treated with miR-34a inhibitor reversed these effects. Intra-articular injection of miR-34a mimic induced cartilage damage, loss of proteoglycan content, and elevated cell death markers; however, miR-34a ASO injections in DMM-induced OA mice was cartilage-protective. Chondrocytes from miR-34a KO mice expressed elevated markers of autophagy, type II collagen, aggrecan, and SIRT1. Plasma, cartilage, and synovial miR-34a was significantly up-regulated in HFD mice compared to LD controls, highlighting the effect of obesity on dysregulated miR-34a expression. Similarly, human plasma miR-34a was sig. up-regulated in obese TKR patients compared to non-obese TKR. Interestingly, intra-articular injections of miR-34a ASO in HFD DMM-induced OA mice protected HFD mice from the severe OA development observed in HFD mice receiving SCO injections.

CONCLUSIONS: Our next goals are to assess the therapeutic effects of genetic miR-34a KO on OA development. Thus, targeting miR-34a may provide therapeutic benefit in delaying or preventing the progression of OA.

Myosin phosphatase Rho-interacting protein regulates DDR1-myosin II interactions and collagen contraction

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Fibrosis affects many major organs after injury and is characterized by dysregulated collagen remodeling that leads to alterations in tissue architecture, function and ultimately, organ failure. DDR1 is a collagen adhesion mechanoreceptor that is associated with fibrotic lesions and is involved in mechanically-mediated matrix remodeling through its associations with non-muscle myosin IIA (NMIIA). The myosin phosphatase Rho-Interacting Protein (MRIP) is a scaffold protein that targets RhoA to the myosin phosphatase complex to regulate myosin phosphorylation and consequently, cellular contraction. We found by tandem mass tag mass spectrometry and confirmed by immunoprecipitation, that MRIP associates with DDR1 during collagen remodeling. Accordingly, we hypothesized that MRIP regulates the interactions of DDR1 with NMIIA to mediate cell migration and tractional remodeling of collagen. The co-localization of MRIP with DDR1, NMIIA or actin filaments was increased by plating on collagen compared with fibronectin ($p<0.01$). Deletion of MRIP by CRISPR/Cas9 in b1 integrin null cells expressing DDR1, inhibited wound closure and blocked the compaction and alignment of collagen fibrils. Cells expressing DDR1 and MRIP applied 3-fold higher traction forces on collagen than cells null for MRIP or cells expressing low levels of DDR1. Deletion of MRIP did not disrupt DDR1-NMIIA association but reduced myosin light chain and DDR1 phosphorylation (Y792). Compared with plating on fibronectin, cells plated on collagen that expressed full-length DDR1 and MRIP formed more abundant, larger and more stable DDR1 clusters based on immunostaining, FRET and FRAP. MRIP null cells expressing DDR1 or cells expressing a non-activating DDR1 mutant formed fewer, smaller and less stable DDR1 clusters. We conclude that the association of DDR1 with NMIIA is involved in DDR1 clustering into collagen adhesions and is regulated by MRIP. Thus MRIP affects the amplitude and kinetics of contractile forces that are applied to collagen-rich ECM and may provide a novel drug target for control of fibrosis.

Free calcium regulates the expression of proteoglycan and collagen in human cartilage

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PURPOSE: Osteoarthritis (OA) is a multifactorial debilitating disease that affects over four million Canadians. Although the mechanism(s) of OA onset is unclear, the biological outcome is cartilage degradation. Cartilage degradation is typified by the progressive loss of extracellular matrix components - aggrecan and type II collagen (Col II) – partly due to the upregulation of catabolic enzymes - aggrecanases a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS-) 4 and 5 and matrix metalloproteinases (MMPs). There is currently no treatment that will prevent or repair joint damage, and current medications are aimed mostly at pain management. When pain becomes unmanageable, arthroplasty surgery is often performed. Interest has developed over the presence of calcium crystals in the synovial fluid of OA patients, as they have been shown to activate synovial fibroblasts inducing the expression of catabolic agents. We recently discovered elevated levels of free calcium in the synovial fluid of OA patients and raised the question on its role in cartilage degeneration.

METHODS: Articular cartilage was isolated from 5 donors undergoing total hip replacement. Chondrocytes were recovered from the cartilage of each femoral head or knee by sequential digestion with Pronase followed by Collagenase and expanded in DMEM supplemented with 10% heat-inactivated FBS. OA and normal human articular chondrocytes (PromoCell, Heidelberg, Germany) were transferred to 6-well plates in culture medium containing various concentrations of calcium (0.5, 1.0, 2.5, and 5.0 mM CaCl2), and IL-1β. Cartilage explants were prepared from the same donors and included cartilage with the cortical bone approximately 1 cm2 in dimension. Bovine articular cartilage explants (10 months) were used as a control. Explants were cultured in the above-mentioned media; however, the incubation period was extended to 21 days. Immunohistochemistry was performed on cartilage explants to measure expression of Col X, MMP-13, and alkaline phosphatase. The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of cartilage was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay, and aggrecan fragmentation was determined by Western blotting using antibody targeted to its G1 domain. Western blotting was also performed on cell lysate from both OA and normal chondrocytes to measure aggrecan, Col II, MMP-3 and -13, ADAMTS-4 and -5.

RESULTS: Ca2+ significantly decreased the proteoglycan content of the cartilage explants as determined by the DMMB assay. The presence of aggrecan and Col II also decreased as a function of calcium, in both the human OA and bovine cartilage explants. When normal and OA chondrocytes were cultured in medium supplemented with increasing concentrations of calcium (0.5-5 mM Ca2+), aggrecan and Col II expression decreased dose-dependently. Surprisingly, increasing Ca2+ did not induce the release of MMP-3, and -13, or ADAMTS-4 and-5 in conditioned media from OA and normal chondrocytes. Interestingly, inhibition of the extracellular calcium-sensing receptor CaSR) reversed the effects of calcium on matrix protein synthesis.

CONCLUSION: We provide evidence that Ca2+ may play a direct role in cartilage degradation by regulating the expression of aggrecan and Col II through activation of CaSR.

Cellular and Molecular Phenotyping and Imaging Pipeline for Target Characterization in Archived Biobank Samples

Invited speaker: Kjetil Ask

McMaster University

The examination and identification of molecular pathways involved in human disease is challenging due to the limited access and conservation method of tissues used for clinical diagnosis. Recent technological advances now allow the examination of semi-quantitative levels of gene expression and co-localization in formalin fixed paraffin embedded tissues stored at room temperature. In this presentation we will demonstrate how we developed a cellular and molecular phenotyping pipeline using archived samples of lung tissue derived from patients diagnosed with fibrotic interstitial lung disease. Dr. Ask will discuss how the archived database of clinically relevant tissues were obtained, clinically categorized and deidentified. He will provide his perspective on the requirement of cross-disciplinary expertise and the associated technological platform required for the identification and quantification of cellular and molecular targets in fibrotic lung disease. Dr. Ask will also discuss the potential application of this platform in translation to pre-clinical models of lung disease and show specific examples characterizing the role of alternatively activated macrophages and endoplasmic reticulum stress in fibrotic lung disease, using automated immunohistochemistry and associated in situ hybridization technology using ACD RNAScope® and BaseScope™ assays to demonstrate molecular phenotyping and target identification.

ABSTRACTS FOR ORAL PRESENTATIONS

Session VI: Clinical and translational research in connective tissue biology

Taking temperature modulation from the bedside to the bench

Invited speaker: Edward Harvey

McGill University

Observations in the clinical world pointed to a new physical modality in bone healing. Discussion of what is happening at the basic science level can help fuel the debate over whether there is a real chance to augment bone healing in humans.

The role of exogenous and endogenous stem cells and biomaterials in bone fracture healing

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INTRODUCTION: Throughout the normal lifespan, bone continuously remodels in response to damage, such as fracture. In healthy individuals, long bone fractures repair through the formation of a cartilaginous callus followed by mineralization and remodeling of the extracellular matrix. However, diseases such as osteoporosis can cause impaired bone healing, increasing the risk of progression to non-repairing defects (fracture non-unions). Promoting the healing of fracture non-unions is a promising target for bone tissue engineering due to the limited success of current clinical treatment methods. There has been significant research on the use of stem cells (adult and pluripotent) with and without biomaterial scaffolds to treat fracture non-unions due to their promising regenerative capabilities. However, the relative roles of transplanted stem cells (exogenous) and stem cells found naturally in the body (endogenous) and their overall contribution to in vivo fracture repair is not well understood, thereby delaying the translation of new tissue engineering therapies to the clinic. The purpose of this research was to determine the interaction between exogenous and endogenous stem cells and biomaterials during bone fracture repair to gain insight into these mechanisms and improve upon bone healing in difficult-to-treat fractures.

METHODS: A 0.7 mm diameter burr-hole tibia fracture was made in a mesenchymal stem/progenitor cell (MSC) lineage tracking mouse (EMCcre-ERT2:tdTomato). In this mouse model the endogenous MSCs permanently emit red fluorescence after induction with tamoxifen. C57BL/6 induced pluripotent stem cells (iPSCs) were pre-differentiated in a Collagen-I scaffold for 5 days and then transplanted directly into the burr-hole fracture at the time of injury. The mice were sacrificed 3 and 7-days post-fracture. Safranin-O and Fast-Green staining was used to assess the degree of bone formation and mineralization, and immunofluorescence was used to identify endogenous MSCs. Low intensity x-ray (Xradia) was used to assess 3D bone structure and healing.

RESULTS: All three groups (untreated controls, collagen alone, and collagen + iPSCs) showed the formation of a boney callus post-injury. Xradia imaging indicated that burr-holes filled with collagen alone demonstrated similar bone formation to controls yet showed an increased presence of endogenous MSCs and proliferating cells as shown by histology/immunohistochemistry. When collagen + iPSCs was transplanted into the burr-hole fracture, this resulted in the absence of endogenous MSCs in the defect site, little evidence of proliferating cells, and minimal bone regeneration.

DISCUSSION & CONCLUSIONS: The introduction of iPSCs inhibited bone regeneration post-fracture. In a normal mouse, bone typically heals well without intervention, so when an external factor, such as iPSCs, is added it may disrupt bone's normal healing mechanisms. Therefore, an osteoporosis disease model will be explored in the future to determine if iPSCs can improve bone regeneration in a model where endogenous regeneration is deficient. To further investigate the role of exogenous iPSCs in bone fracture healing, green-fluorescent protein tagged iPSCs will be used to directly trace their activity in vivo. A better understanding of the biological mechanisms through which bone heals will provide necessary knowledge for developing more effective stem cell therapies to treat clinical non-union fractures.

Enhanced bone repair after fracture priming

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INTRODUCTION: Bone repair starts with a localized inflammatory response during which immune and vascular precursor cells are attracted to the defect. Our previous work has demonstrated that the immune system through the action of macrophages and mast cells regulate angiogenesis, osteoclast activity, and the inflammatory environment during bone healing. The goal of this project is to use a mouse model of bone repair to determine if priming immune cells to recognize broken bone in a first fracture accelerate bone repair in a second fracture sustained shortly after.

METHODS: Bone repair in skeletally mature Bl6 mice was quantified in 2mm cortical window defects drilled in the femur. Mice were randomly assigned to one of two treatment groups: 1) Double fracture where the first defect was drilled on the left leg, and 14 days later, a second defect was drilled on the right one; or 2) control where both defects were drilled at the same time. The mice were allowed free ambulation and access to food and water for 14 or 56 days before euthanasia and femur harvest. The amount and quality of bone and revascularization of the defect were quantified using micro CT. Mast cell, osteoblast, osteoclast, vascular endothelial cell, and macrophage distribution pattern and activity were assessed by histology. Comparisons were made by T-tests at the 95% confidence level.

RESULTS: In a standardized region spanning the defect, at 14 days of healing we found no differences in bone quantity between groups. Significantly more and larger mast cells were found in the periosteum of the double fracture mice, along with more osteoclast activity and more macrophages, compared to controls. Blood vessels in the double fracture group also seemed to be more organized. We found no difference in osteoblast activity between groups. At 56 days of healing, all mice exhibited bridging of the defects, with significantly more bone tissue with less difference in the mineral content between the repair tissue and the healthy bone in the double fracture group. This finding was matched by a significantly higher number of osteocyte lacunae in the repair tissue in the double fracture group. Interestingly, the differences at 56 days postoperative were similar between mast cell competent WT and mast cell deficient Cpa3Cre/+ mice, suggesting a mechanism independent of mast cell activity.

CONCLUSIONS: Taken together, the results suggest a more intense inflammatory reaction during the early stages of repair in the double fracture group which leads to enhanced bone repair after 8 weeks of healing. Ongoing histological analysis will help identify the spatial pattern of distribution of immune, vascular and skeletal cells at this time point. Our results may have implications for the development of immune cell-based therapies to promote bone healing.

Aberrant sialoglycan patterns facilitate 3D multicellular spheroid and xenograft tumor formation

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Multicellular tumor spheroids are now at the forefront of cancer research, designed to mimic tumor-like developmental patterns in vitro. Tumor growth in vivo is known to be highly influenced by aberrant cell surface specific sialoglycan structures on glycoproteins. Aberrant sialoglycan patterns that facilitate spheroid formation are not well defined. Here, matrix-free spheroids from human breast, pancreatic and prostate cancer cell lines and their respective chemoresistant variants were generated using an unique cyclic Arg-Gly-Asp-D-Phe-Lys peptide modified with 4-carboxybutyltriphenylphosphonium bromide (cyclo-RGDfK (TPP)) induced self-assembly platform. The cyclo-RGDfK(TPP) peptides mimic the natural extracellular matrices (ECM) protein's ability to induce cell aggregation via α5β1 integrin. We used the cyclo-RGDfK(TPP) approach to biochemically induce cell aggregation and compaction, transmogrifying monolayer cancer cells into tumor spheroids. MCF-7 and PANC-1 cells, and their drug-resistant cancer cell lines (MCF-7 TMX, PANC1-GemR) express different sialic acid content, which influenced their ability to form spheroids under cyclo-RGDfK(TPP)-induced self-assembly. Cancer cell aggregation and compaction correlates with the presence of α-2.3- and α-2,6-sialic acid cell surface residues to form spheroids under cyclo-RGDfK(TPP)-induced self-assembly and xenograft tumors. Removal or blockage of SA inhibited cell aggregation. Neuraminidase inhibitor, oseltamivir phosphate, enhanced cell aggregation and promoted compaction of cell aggregates. Future studies should build upon these findings and explore alternate and novel methods to target the cancer cell glycome and the unique sialylation patterns of the adhesion molecules involved in spheroid formation and tumor progression.

Natural senolytics to relief back pain

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INTRODUCTION: Intervertebral discs (IVDs) degeneration is one of the major causes of back pain. Upon degeneration, the IVDs tissue become inflamed, and this inflammatory microenvironment may cause discogenic pain. Cellular senescence is a state of stable cell cycle arrest in response to a variety of cellular stresses including oxidative stress and adverse load. The accumulation of senescent IVDs cells in the tissue suggest a crucial role in the initiation and development of painful IVD degeneration. Senescent cells secrete an array of cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP). The SASP promote matrix catabolism and inflammation in IVDs thereby accelerating the process of degeneration. In this study, we quantified the level of senescence in degenerate and nondegenerate IVDs and we evaluated the potential of two natural compounds to remove senescent cells and promote overall matrix production of the remaining cells.

METHODS: Human IVDs were obtained from organ donors. Pellet or monolayer cultures were prepared from freshly isolated cells and cultured in the presence or absence of two natural compounds: Curcumin and its metabolite vanillin. Monolayer cultures were analyzed after 4-days and pellets after 21 days for the effect of senolysis. A cytotoxicity study was performed using Alamar blue assay. Following treatment, RNA was extracted, and gene expression of senescence and inflammatory markers was evaluated by real-time q-PCR using the comparative ΔΔCt method. Also, protein expression of p16, Ki-67 and Caspase-3 were evaluated in fixed pellets or monolayer cultures and total number of cells was counted on consecutive sections using DAPI and Hematoxylin. Proteoglycan content was evaluated using SafraninO staining or DMMB assay to measure sulfated glycosaminoglycan (sGAG) and antibodies were used to stain for collagen type II expression.

RESULTS: We observed 40% higher level of senescent cells in degenerate compare to the non-degenerate discs form unrelated individuals and a 10% increase when we compare degenerate compare to the non-degenerate discs of the same individual. Using the optimal effective and safe doses, curcumin and vanillin cleared 15% of the senescent cells in monolayer and up to 80% in pellet cultures. Also, they increased the number of proliferating and apoptotic cells in both monolayer and pellets cultures. The increase in apoptotic cell number and caspase 3/7 activity were specific to degenerate cells. Following treatment, mRNA expression levels of SASP factors were decreased by 4 to 32-fold compared to the untreated groups. Senescent cell clearance decreased, protein expression of MMP-3 and -13 by 15 and 50% and proinflammatory cytokines levels of IL-1, IL-6 and IL-8 by 42, 63 and 58 %. Overall matrix content was increased following treatment as validated by an increase in proteoglycan content in pellet cultures and surrounding culture media.

DISCUSSION: This work identifies novel senolytic drugs for the treatment of IVD degeneration. Senolytic drugs could provide therapeutic interventions that ultimately, decrease pain and provide a better quality of life of patients living with IVDs degeneration and low back pain.

Strong bones across the lifespan: role of mechanical loading

Invited speaker: Saija Kontulainen

University of Saskatchewan

In this lecture, Dr. Kontulainen will discuss bone adaptation to physical activity with a specific focus on evidence from clinical studies employing advanced imaging, objective measures of physical activity and assessments of muscle-bone interplay. The lecture will review levels of evidence with examples spanning from observational studies to randomized controlled exercise trials, targeting both growing and aging skeleton. Findings will be discussed in relation to theoretical bases of bone adaptation to loading stimulus, as per the Mechanostat model. Physical activity interventions tailored for individuals at risk of fracture and future patient-oriented research directions will be discussed.

ABSTRACTS FOR ORAL PRESENTATIONS

Session VII: Imaging, genetics, and high-throughput screening of connective tissues

Pre-clinical and clinical imaging of the musculoskeletal system

Invited speaker: David Holdsworth

Western University

Biomedical imaging plays a key role in pre-clinical and clinical investigations of the musculoskeletal system. Recent technical advances have significantly expanded the capabilities for imaging soft tissue and bone, with research applications related to arthritis, trauma, sports medicine, and arthroplasty. This talk will focus on some of the work that is being carried out within our group and elsewhere in the area of quantitative musculoskeletal imaging. Basic-research applications include real-time live-cell microscopy and dual-energy micro-computed tomography imaging of microvasculature and bone. Micro-CT can also be applied effectively in cadaveric imaging of the spine and lower extremities, including micro-imaging of the knee joint during computer-controlled loading protocols. Quantitative clinical applications include radiostereometric analysis (RSA) of prosthetic components, following arthroplasty, and parametric mapping of soft tissue using MRI. Future applications for biomedical imaging of the musculoskeletal system include image-based finite-element models and fabrication of patient-specific prosthetic components, as well as dynamic imaging of bones and joints under normal physiological loads. Recently developed CT systems provide the capability for routine imaging of extremities, including standing imaging of the foot, knee, and ankle. Medical imaging has come a long way since Roentgen's first image of his wife's hand over 120 years ago!

Known and novel circulating microRNAs are uniquely expressed in knee osteoarthritis

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OBJECTIVES: MicroRNAs are small non-coding RNAs that repress expression of target genes. Circulating microRNAs have emerged as promising biomarkers for many diseases. There are still no biomarkers that can be used in a therapeutic, prognostic, or diagnostic manner for osteoarthritis (OA). Our lab was the first to perform global array screening of microRNAs in OA, but next generation sequencing (NGS) is a superior method for identifying all known and novel microRNAs in a biological sample. The objective of this study is to use next generation sequencing to identify microRNAs in plasma samples from patients with knee OA. This technology has the sensitivity and specificity to detect microRNAs that are unique to various cohorts, including early knee OA versus late knee OA.

METHODS: Plasma samples from 50 late OA patients, 41 early OA patients, and 50 healthy donors were subjected to NGS of microRNAs. Cohorts were defined based on Kellgren-Lawrence radiographic grades 3 or 4 for late OA, grades 0 or 1 for early OA, and no history of musculoskeletal disease for healthy donors. Following microRNA extraction, libraries were prepared using the Qiagen QIAseq microRNA Library Kit, and sequenced on the Illumina NextSeq550 sequencer. Data analysis included collapsing of unique molecular indices to control for library amplification bias. Following alignment to miRBase 22, microRNA counts were generated and the final list of differentially expressed microRNAs were identified using negative binomial count modelling with the edgeR (v3.18.1) package in R (v.3.2.2).

RESULTS: Our analysis revealed a clear separation of early OA samples from late OA samples. The most differentially expressed microRNAs were identified based on false discovery rate less than 0.05, log counts per million greater than 2, and log fold change greater than 1.5. When comparing early OA samples to late OA samples, 116 differentially expressed known microRNAs were identified. Hierarchical clustering of these microRNAs revealed a distinct pattern where a subset of microRNAs was upregulated only in early OA samples. Among these are novel putative microRNAs that have not previously been characterized. These novel microRNAs were discovered using miRDeep2 (v2.0.0.8) based on predicted secondary structure and lack of homology with microRNAs in mouse.

CONCLUSIONS & FUTURE DIRECTIONS: This study demonstrates that NGS is a useful tool for identifying known and novel circulating microRNAs in OA which may be valid and reliable biomarkers for improving detection and treatment. Next steps include target gene prediction and pathway analysis to identify potential disease mechanisms that are governed by these microRNAs.

Can micro-computed tomograhy detect bone adaptations from ex vivo culture?

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OBJECTIVE: Ex vivo culture allows maintenance of viable tissue samples in a bioreactor. This type of experiment is of interest to study the influence of mechanical loading on bone remodelling (David 2008, Endres 2009, Vivanco 2013). Compared to in vivo testing, ex vivo bone cultures have the advantage that they allow a better control of the environment and experimental conditions applied to the sample. However, a difficulty associated with this type of experiment is the capability to quantify sample's adaptation after the culture period. Micro-computed tomography (CT) is a standard measurement method used to quantify density and structural parameters of the bone which are commonly used to assess bone quality. Therefore, the aim of this project was to determine if bone adaptations resulting from ex vivo culture can be detected with micro-CT imaging.

METHODS: Two ilia were obtained from a seven year old bovine. The ilia were processed within 3 hours after the death of the animal. Six rectangular cuboid trabecular bone samples were extracted from the ilia (H= 10 mm, W=14 mm, D= 10 mm). The samples were cultured in a bioreactor for 14 days. Each day, samples were submitted to dynamic mechanical loading of 4000 microstrain (300 cycles, 1 Hz) to stimulate bone adaptation. The samples were imaged by micro-CT (Skyscan 1176) at a resolution of 8 μm (65 kV, 385 uA), before and after the culture period to quantify the changes after bone adaptation. The images were reconstructed and segmented using the software inherent to the scanner (NRecon version 1.6.8.0, CT Analyzer version 1.12.11.0). The change in density measurement and structural parameters due to culturing were compared with the same measurements made on a control sample which had not been cultured. The control sample was also used to determine the variability of the analysis method by repeating the analysis four times.

RESULTS: The change in density measurement, as well as the structural parameters following 14 days of culture was higher than the change obtained for the control sample that was not cultured. The mean mineral density of the cultured samples varied by 0.047 ± 0.023 g/cm3 compared to a measurement variation of 0.016 ± 0.002 g/cm3 when repeating the analysis with the control sample. The trabecular thickness (Tb.Th) and the trabecular separation (Tb Sp.) of the cultured samples varied by 0.013 ± 0.005 and 0.009 ± 0.012 mm respectively compared to 0.001 ± 0.000 and 0.001 ± 0.000 mm for the control sample. Consequently, the changes in the measurements obtained were due to culturing and not to the acquisition and image analysis.

CONCLUSION: This project confirms that X-ray micro-CT is an effective measurement method to detect bone adaptation resulting from ex vivo culture. The use of micro-CT appears to be an adequate method to quantify and document sample changes during ex vivo culture. Eventually, this measurement tool might be used during ex vivo culture to have a better understanding of bone adaptation from diseases such as osteoporosis or other bone remodeling processes such as osteointegration.

Usp53, a PTH target regulating bone turnover and mesenchymal stem cell differentiation

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INTRODUCTION: The daily injection of parathyroid hormone (PTH) increases bone mass and protects against osteoporotic fractures, however a complete picture of its mechanism of action is still missing. We have shown that PTH induces the phosphorylation of the DNA-binding protein Nascent polypeptide associated complex And Coregulator alpha (aNAC), leading to nuclear translocation of aNAC and activation of target genes.

PURPOSE: The aim of the study is to identify and characterize novel target genes regulated by PTH-activated aNAC in osteoblasts.

METHODS: We performed Chromatin Immunoprecipitation with deep sequencing (ChIP-Seq) against aNAC with parallel RNA-sequencing in PTH-treated osteoblast cells. The biological role of candidate genes was studied in vitro in osteoblastic cells and bone marrow stromal cells (BMSCs) cultures using shRNA-mediated knockdown techniques and in vivo by deleting candidate genes using a CMVCre driver.

RESULTS: This strategy has identified the Ubiquitin-Specific Peptidase 53 (Usp53) as a candidate gene target of PTH-activated aNAC in osteoblasts. Little is known about the biological function of Usp53 in bone biology. PTH treatment of osteoblast cells increased the mRNA expression of Usp53 by 3-fold as compared to vehicle-treated cells but PTH-driven transcriptional induction of Usp53 was completely blunted following aNAC knockdown. The global deletion of Usp53 in vivo led to a brittle bone phenotype, assessed by 3-point bending and identation mechanical testing. Usp53(-/-) femurs and tibias showed an increased tendency to yield and break as compared to control samples. Osteogenic cultures of Usp53(-/-) BMSCs revealed elevated levels of RANKL. Besides, Usp53(-/-) tibial RNA showed an increase in osteoclast-related markers as compared to controls. Serum bone turnover markers including CTX-1 and P1NP were also elevated. Usp53(-/-) BMSCs cultures showed decreased adipogenesis, mainly due to decreased levels of Pparγ. Proteomics has revealed a novel interaction of USP53 with TAK1-TAB1/2 complex proteins. Our data showed that USP53 is critical for the TAK1-TAB1/2 complex assembly. The absence of USP53 disrupted JNK-MAP kinase signaling downstream of the TAK1-TAB complex.

CONCLUSION: This is the first report characterizing the physiological role of Usp53 in bone. Our data suggest that Usp53 affects bone homeostasis and mass in vivo, mainly by regulating bone remodeling and osteoclastogenesis. The novel interaction of USP53 with the TAK1-TAB1/2 complex; a TGF-β/BMP-regulated complex orchestrating the crosstalk between osteogenesis and adipogenesis, suggests that Usp53 is a regulator of lineage fate decisions.

The non-invasive modelling of body-wide inflammation in Duchenne muscular dystrophy

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BACKGROUNG: Duchenne muscular dystrophy (DMD) is a neuromuscular disorder caused by protein dystrophin loss within various tissues—notably cardiac and skeletal muscles. DMD is characterized by progressive muscle degeneration that is accompanied by chronic inflammation, fibrosis, and ischemia, before death by cardiac or respiratory failure. While there is no known cure, drug or experimental muscle regenerative therapies can minimize symptoms and prolong ambulation. However, as some skeletal muscle regenerative therapies impair cardiac function, invasive muscle biopsies—current gold standard for DMD assessment—may be unsuitable for tracking DMD therapeutic outcomes, as they are both painful and localized to the specific muscle segment despite DMD being a systemic disease. Thus, this study aimed to develop an imaging protocol to non-invasively model DMD progression without the need for individual tissue isolation. As our group already demonstrated that inflammation levels correlate with disease severity levels in DMD murine hind-limbs, we hypothesized that this is possible through 18F-FEPPA positron emission tomography (PET) and dynamic contrast-enhanced computed tomography (DCECT) by quantifying changes in inflammation and perfusion parameters respectively.

METHODS: Three murine models—with muscle pathologies resembling DMD-diagnosed humans— were used to represent increasing DMD severity (mild, moderate, severe) within three age groups (3–5, 8–10, and >15 weeks old). Each mouse were imaged with uPET, and associated biodistribution and autoradiography analyses using 18F-FEPPA—a radiotracer targeting mitochondrial translocator protein (TSPO) overexpressed on activated macrophages. Changes in perfusion parameters (e.g. blood flow, blood volume), whose restriction is associated with fibrosis and thereby DMD, is measured through DCECT to assess DMD progression. After imaging, each mouse's mixed, fast- and low-twitch skeletal muscle (e.g. gastrocnemius, soleus, tibalis anterior), and heart were collected for TSPO quantification using immunohistochemistry. Tissues were also stained with known inflammation and fibrosis markers (e.g. IL-6/CD68) and quantified using ImageJ software.

RESULTS: Moderately severe DMD mice imaging demonstrated global elevation in TSPO-PET tracer in the heart and gastrocnemius compared to age-matched wild-type control, which co-localized with increased tracer uptake and fluorescence intensity in autoradiography and immunohistochemistry findings respectively (n=3). Further immunohistochemistry findings of heart and gastrocnemius tissue sampled from mice models of increasing DMD severity also showed progressively increasing levels of TSPO expression. Exploratory biodistribution data also showed heightened uptake of 18F-FEPPA across different tissue types—particularly in the heart and gastrocnemius.

CONCLUSION: 18F-FEPPA PET/DCECT imaging showed concurrent inflammation levels in cardiac and skeletal muscles of DMD, which progresses with disease severity. These data are the first to model DMD via a whole-body in-vivo system, illustrating the advantage of 18F-FEPPA PET as a useful tool to non-invasively detect DMD progression simultaneously in multiple affected organs, increasing the possibility to propose early intervention prior to severe muscular or cardiac damage. Considering the tight coupling of both skeletal and cardiac muscle inflammation, further investigation into the existence of a link between cardiac and skeletal muscle degeneration using inflammation in a dystrophic disease should be investigated. It also offers a novel approach to assessing efficacy of DMD treatments, which may now include anti-inflammatory therapies.

Bone cell cross talk under mechanical loading

Invited speaker: Lidan You

University of Toronto

Bone is able to adapt its composition and structure in order to suit its mechanical environment. Osteocytes, bone cells embedded in the calcified matrix, are believed to be the mechanosensors and responsible for orchestrating the bone remodeling process. However, detailed cellular and molecular mechanism underlying osteocyte mechanobiology is not well understood. In this talk, osteocyte intracellular response under pressure and shear stress were presented. Furthermore, inter cell-population communications under mechanical loading and its implication in bone disorder management such as bone metastasis prevention will be discussed.

Bone metastases are common and severe complications of cancers. Metastasized cancer cells have devastating impacts on bone quality due to their ability to alter bone remodeling. Exercise, often used as an intervention for patients suffering from cancer, regulates bone remodeling. We hypothesize that mechanical loading may regulate bone metastases via osteocyte signaling.

Results from our studies suggest that osteocytes are highly sensitive to mechanical loadings. Mechanical loading and high frequency and low magnitude loading can induce signals inhibit bone resorption and promote bone formation. Loading on osteocyte also have major impact on cancer cell migration and invasion, provides insights into the impact of exercises on bone metastases.

Connective tissues in development and disease

T1.

Circadian clock regulation of molecular mechanisms underlying load-induced bone formation

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Bone adapts to mechanical loading via (re)modeling, a process involving bone-forming osteoblasts, bone-resorbing osteoclasts, and mechanosensing osteocytes. Circadian rhythm is thought to influence bone formation and resorption, but little is known concerning the mechanism(s) of action. We investigated if time of loading influences gene expression to provide insights into whether load-induced bone formation in mice is regulated by circadian oscillator mechanisms functioning in bone tissue. The left tibia of young female mice underwent a single session of dynamic compressive loading at ZT2 or ZT14. Mice were euthanized and dissected 8h or 24h later, at ZT2, ZT10, ZT14 or ZT22. We found with qPCR that *Clock*, *Per1* and *Per2* were expressed in bone. In nonloaded, right tibiae, we observed a significant effect of time for expression of *Per2* and bone-specific, mechanically-responsive gene *Sost* with peaks at ZT14 and ZT22, respectively. Loading at ZT2 led to a significant increase in *Sost* expression after 8h while loading at night led to a significant decrease after 24h. These data suggest the time of loading influences the expression of mechanically-responsive genes. These data could help determine at what time of day patients should undergo physical exercise to enhance load-induced bone formation.

T2.

Systematic review of the effects of space travel on bone health in animals

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Bone loss is one of the unsolved problems arising in long duration spaceflights. Our objective was to systematically identify all the studies reporting measurements related to bone health in animals that experiences microgravity during a spaceflight. The electronic databases Medline, Embase, PubMed, BIOSIS Previews, and Web of Science were searched for studies presenting data for measurements of bone health in animals that traveled to space. Original search identified 14941 unique papers, from which after title/abstract screening 1105 manuscripts were identified as describing spaceflight-related changes in animal physiology. The vast majority of studies described changes in rats (657/1105), followed by mice (116/1105) and primates (94/1105). Additional species included fish, birds, frogs, and newts (~30 manuscripts/species), dogs (16 manuscripts), snails, gerbils and insects (<10 manuscripts/species) as well as ~30 manuscript for other species. The experiments on rats were mostly performed in 1980s-1990s, while mice became a preferred model in the recent decades. Various physiological factors related to bone health have been investigated, including muscle function (278/1105), development (134/1105), and metabolism (107/1105). Next, we focused on the full text screening of studies quantitatively describing changes in bone health only in three main animal species that traveled to space: rats, mice and primates. From 313 articles, we identified 129 manuscripts reporting data from 48 independent experiments, which included data on bone composition, structure, density, and quality (87 manuscripts), bone cell activity and gene expression (43 manuscripts), as well as changes in bone marrow (15 manuscripts) and calcium homeostasis (4 manuscripts). Our study demonstrates that an active area of research on animal adaptation to spaceflight has generated sufficient number of publications on rats, mice and primates to permit meta-analytic synthesis of microgravity-induces changes in bone-related measures in these species.

T3.

Digital infrared thermography reduces length of stay for burned patients

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BACKGROUND: Burns are the third most common type of injury worldwide and account for more than 300,000 annual deaths, especially among children in the developing world. Burn care is traditionally considered expensive care with inhospital costs per patient exceeding $80,000. Moreover, when complications associated with burn injuries arise, treatment costs grow exponentially. Thus, adequate early burn management is crucial to avoid excessive costs and increases in patient morbidity and mortality. Despite this, the initial burn wound assessment has been shown to be inaccurate in up to 30-50% of cases. To overcome this limitation, our team recently developed a prediction model that uses the thermal characteristics of the wound to predict the treatment modality most likely to benefit the patient. Our prediction algorithm demonstrated an accuracy of over 85%, thereby suggesting that its use could help streamline patient treatment, avoid prolonged hospitalizations, reduce complications, and cut treatment associated costs. The objective of the present study is to conduct a randomized clinical trial to confirm this hypothesis.

METHODS: After consenting to participate in the study and admission into a burn care unit, thermal imaging of patients with burns in 10-45% of the body surface area was obtained using an imaging camera for smartphones (FLIR ONE pro) during their initial assessment. Afterwards, the patients were randomized to receive either the usual care pathway (control group) or burn care dictated by the prediction algorithm (experimental group). After discharge, length of stay (LOS) and complication rate between groups was compared. This trial is registered at clinicaltrials.gov with registry number NCT03876340.

RESULTS: A total of 64 patients have been enrolled in the study so far, 31 in the control and 33 in the experimental group. Except for burn body surface area (20% control vs. 13% experimental, $p < 0.001$), all demographic and clinical characteristics of the patients were similar across groups. LOS was significantly reduced in the experimental group (median of 11 vs. 21 days, $p < 0.001$), while complication rate was not different between groups (12% control vs. 9% experimental, $p = 0.81$). After multivariate adjustment of confounding factors, treatment group, age, burn etiology, and burn surface area remained associated to LOS. In the control group, the prediction algorithm correctly predicted the patient's treatment in 80% of the cases (weighted Kappa = 0.87).

CONCLUSION: Our results show that a relatively simple digital infrared thermal imaging protocol can greatly impact the LOS of burned patients in a burn care unit. The study is still underpowered to detect difference in the complication rates, but we believe there is a trend towards also reducing them by using the prediction algorithm. The study is still ongoing and we are planning to enroll at least 60 patients per treatment group before doing a cost-effectiveness analysis.

T4.

Targeting receptors for curcumin and vanillin in painful degenerating intervertebral discs

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BACKGROUND: Pain in intervertebral disc (IVD) degeneration is one of the most common disabilities in young and middle-aged individuals. Although very common, treatment is costly and limited; there are no early treatments available and for patients with advanced stages of the disease invasive surgery is the only option to relieve the pain. A major problem with available treatment options, is that there is limited understanding regarding the early onset and progression of painful IVD degeneration. More specifically, it is unclear how pain and degeneration are initiated and how they can be prevented. Current evidence suggests that changes in the biomechanical properties of degenerating discs are associated with matrix fragmentation, inflammation and pain. Moving away from costly conventional pharmacotherapy which often has significant negative side effects, there is now interest in natural plant products with anti-inflammatory and regenerative properties such as Curcumin (diferuloylmethane) and Vanillin; a metabolite of Curcumin. The overall objective of this project is to evaluate the potential of Curcumin and Vanillin to reduce inflammatory mediators and proteases in cells from painful degenerating human IVD. Having both anti-inflammatory and anti-oxidant properties in chondrocytes, we hypothesize that Vanillin will reduce the level of cytokines and proteases present to reduce spinal pain and delay the need for surgical interventions in patients with low back pain. To address this hypothesis, we used painful degenerating IVD cells obtained from patients undergoing surgery for low back pain to; 1) quantify the potential of Vanillin to reduce cytokines and proteases.2) examine the expression profile of known target receptors for Vanillin at steady state and following treatment.

METHODS: cDNA was prepared from RNA extracted from painful degenerating IVD cells. The cells were either be left untreated or treated with Vanillin. By qPCR, we measured the relative expression level (ΔΔCt)of IL-1ß, IL-8, TNFa and NGF to GAPDH (housekeeping gene). To quantify protein cells were treated as done for qPCR however, the expression of these cytokines were assessed by Quantification was be done using ImageJ; with optical density compared between treated vs. non-treated cells. Using the same cell populations as described in aim 1, we will measure the relative expression level (ΔΔCt)of receptors; CX3CR, Mu and Delta opioid receptors, 5-HT(1A), TRPV-1, TLR 1, 2, 4 and 6, IL-1 receptor I and II, TNF-receptor I and -II at baseline and after treatment with vanillin to the housekeeping gene GAPDH via qPCR.

RESULTS: Vanillin reduced the levels of cytokines and proteases present at the transcript and protein level in cells from surgical specimens of patients undergoing surgery for low back pain that are treated versus those that are not treated. Also, it is anticipated that the pain degenerating IVD treated cells will have significantly less receptor expression at the transcript level when compared to the to the non-treated cells from pain suffering patients. Our initial results have already shown a significant decrease in pain mediators NGF and IL-8 at the protein level through immunohistochemistry.

T5.

The role of circ-Itga9 in cardiac remodeling

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Cardiac disease is a major death cause worldwide and so far, there is no effective method to prevent it. Circular RNA is a novel type of endogenous noncoding RNA (ncRNA), which has become a research hotspot in recent years. Recent studies have shown that circular RNAs may play important physiological and pathological roles in cardiovascular diseases. Our RNA sequencing data showed that circ-Itga9, a circular RNA generated from a gene (Integrin alpha 9) that encoded an adhesion molecule, was significantly up-regulated in in patients with tetralogy of fallot (TOF), aortic stenosis (AS), mitral stenosis (MS), as compared with normal myocardium tissues, suggesting a potential role in cardiac remodeling processes. Increased expression of circ-Itga9 was confirmed in large pools of specimens from TOF, AS, MS and heart donation samples. *In vitro*, we detected characteristics of cardiac remodeling in cardiomyocytes and cardiac fibroblasts by overexpression of circ-Itga9 with a circ-Itga9 expression construct. *In vivo*, we found exaggerating cardiac remodeling following mouse transverse aortic constriction (TAC) when the mice were injected with the circ-Itga9 expression construct. To further investigate the underlying mechanisms, we identified potential circ-Itga9 binding proteins using circular RNA precipitation process followed by Liquid chromatography tandem-mass spectrometry (LC-MS/MS). Through double confirming MS results using circular RNA pull down/precipitation assays, we found that overexpression of circ-Itga9 could bind to more tropomyosin 3 (TPM3) compared to vector control. Specific binding sites between circ-Itga9 and TPM3 were identified using computational algorithms and further investigated using site-directed mutagenesis. Taken together, our study outlined the novel role of circ-Itga9 in cardiac remodeling and its underlying mechanisms. The novelty of our study is potentially amenable for further preclinical and translational development.

T6.

L-plastin and PRDX2 are osteoclastogenic factors secreted by actively proliferating erythropoietic cells

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Bone and bone marrow are parts of the same organ and are known to exhibit active crosstalk. Hematopoietic disorders such as hemolytic anemias commonly result in bone loss. We have previously reported that breast cancer cells actively proliferating in bone marrow can stimulate osteoclast formation, and identified peroxiredoxin (PRDX) 4 and L-plastin as critical mediators of this process. The goal of this study was to examine if hematopoietic cells can trigger osteoclastogenesis in a similar manner. Conditioned medium (CM, 10%) from primary mouse erythroblasts and K562 erythroleukemia cells stimulated osteoclastogenesis when added to osteoclast precursors (primary mouse bone marrow-derived (BM) or RAW264.2) primed with RANKL (50ng/ml) for 2 days and osteoclastogenesis was assessed on day 5-7. Using immunoblotting and mass spectrometry, we identify L-plastin and PRDX2 as the factors produced by erythroid cells. Treatment of RANKL primed BM and RAW 264.7 osteoclast precursors with recombinant L-plastin and PRDX2 confirmed that these two factors can induce osteoclast formation from RANKL-primed precursors. Since L-plastin and PRDX2 are known as cytosolic proteins, we hypothesized that they can be transported by exosomes. Exosomes were isolated from CM of proliferating K562 cells by differential centrifugation. Transmission electron microscopy imaging confirmed the size and shape of exosomes. Immunoblotting showed that exosomal fraction, identified by specific markers transferrin2 and TSG101, also contained Lplastin and PRDX2. In vivo, we modeled acute blood loss by withdrawing 10% of total blood volume through saphenous vein of 6 weeks old C57Bl/6 female mice. Transient anemia was confirmed by decrease in hematocrit on days 1-3, increase in expression of hematopoietic factors GATA1 and Epo-R starting at day 1, and increase in spleen weight at days 2-3. Importantly, protein expression of L-plastin and PRDX2 transiently increased with a time course corresponding to a decrease in hematocrit; while osteoclast markers, calcitonin receptor and cathepsin K, were elevated in day 2-3 BM of anemic mice compared to sham control.

Taken together, our data suggest that anemia-induced bone marrow erythropoiesis leads to upregulation of L-plastin and PRDX2, which mediate osteoclastogenesis. Increased bone resorption in turn leads to expansion of bone marrow cavity further supporting blood production.

T7.

Retro-Inverso Tat-Beclin-1 induces synovial fibrosis and does not protect cartilage from degeneration in a mouse model of OA

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PURPOSE: Beclin-1 is a component of the autophagy pathway necessary for formation of autophagosomes, contributing to autophagy-mediated cellular homeostasis. Enhancing autophagy through inhibition of mTOR activity, either via genetic deletion in chondrocytes or intra-articular injection of rapamycin, attenuates progression of surgically-induced models of osteoarthritis (OA). Retro-inverso TAT-Beclin-1 is a cell-permeable peptide which competes for binding to the endogenous Beclin-1 inhibitor GAPR-1, thus promoting autophagy. It is unknown whether activation of Beclin-1 is sufficient to protect joints from osteoarthritis progression. For this study, we sought to determine if retro-inverso TAT-Beclin-1 could attenuate OA progression in a surgically- induced mouse model.

METHODS: Eight-week old C57BL/6 mice underwent destabilization of the medial meniscus (DMM) surgery to induce OA, or sham surgery as a control. Mice were injected intra-articularly with retro-inverso TAT-Beclin-1 (0.4-2 mg/kg in 5µl) twice weekly for 2 or 9 weeks. Mice were sacrificed at 10-weeks post-surgery. Knee joints were stained with Safranin-O/Fastgreen to evaluate cartilage degeneration and Masson's trichrome to determine degree of synovitis using OARSI scoring for mice. Sections were stained for α-SMA (myofibroblast) and CD45 (hematopoietic-origin cell) to evaluate changes in markers of fibrosis and inflammation, respectively.

RESULTS: As opposed to the effects of mTOR deletion in cartilage or rapamycin treatment in joints, injection of retro-inverso TAT-Beclin-1 into knee joints of mice with DMM-induced OA had no effect on the degree of articular cartilage degeneration in the tibia or femur as compared to PBS-injected controls regardless of dose or injection regimen. However, in both sham and DMM mice, retro-inverso TAT-Beclin-1 treatment induced a pronounced thickening of the synovium with increased cell numbers and collagen deposition compared to PBS-treated mice. The synovial thickening effect of retro-inverso TAT-Beclin-1 was dose dependent. The increased number of synovial cells did not show substantial expression of α-SMA+ or CD45+ cells, suggesting the increased number of cells and matrix in the synovium was independent of myofibroblast differentiation or inflammatory influx.

CONCLUSIONS: Contradictory to our expected results, retro-inverso TAT-Beclin-1 did not attenuate cartilage degeneration. Rather, it promoted substantial synovial thickening that likely involved cell proliferation and collagen deposition. This severe fibrotic phenotype appears independent of myofibroblast differentiation or inflammation, normally associated with typical fibrotic responses. We are currently investigating the cell type/layer contributing to the thickening of the synovium through in vivo proliferation assays.

T8.

MicroRNA 27b-3p: Role in extracellular matrix regulation in osteoarthritis synovial fibroblasts

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PURPOSE: Synovial fibrosis is a key pathological event that contributes towards joint destruction, pain and stiffness associated with osteoarthritis (OA). Fibrosis is generally characterized by excessive extra-cellular matrix (ECM) accumulation, however, endogenous mechanisms of synovial fibrosis in OA are not well understood. We have previously shown that microRNA27b-3p (miR-27b-3p) is abundantly expressed in OA synovium and elevated in the synovial fluid of late-stage (Kellgren-Lawrence; KL score 3-4) compared to early-stage (KL score 1-2) radiographic knee OA patients (Y-H. Li, G. Tavallaee, et al. 2016). Since the role of miR-27b-3p in OA synovial pathology has never been studied; we investigated if miR-27b-3p plays any role in ECM regulation in the OA synovium.

METHODS: Primary OA synovial fibroblasts (SFbs; major cell type in the synovium) were transfected with miR-27b-3p mimic, profiled by qPCR array of 84 cell adhesion and ECM markers, and validated by quantitative-PCR. SFbs were further transfected with miR-27b-3p mimic, followed by ADAMTS8 siRNA silencing. Transwell was used to examine the effect of miR-27b-3p on OA SFbs migration. For in vivo studies, mice were subjected to DMM model of OA to determine the expression of miR-27b-3p during the time course of OA development in knee joint using in situ hybridization (ISH).

RESULTS: ECM-specific qPCR-array and validation of responsive genes revealed that miR-27b-3p mimic significantly upregulates the expression of key ECM genes including pro-collagen Type-I (COL1A1), A disintegrin and metalloproteinase with thrombospondin motifs8 (ADAMTS8), Thrombospondin1 (THBS1), Fibronectin1 (FN1), Collagen 5-A1 (COL5A1) and Collagen 14-A1 (COL14A1) in OA SFbs. Furthermore, miR-27b-3p mimic resulted in a marked increase in type-I collagen protein expression, which was suppressed by miR-27b-3p inhibitor, suggesting that miR-27b-3p may play a key role in the regulation of key ECM components in OA SFbs. As ADAMTS8 was the most responsive gene to miR-27b-3p, we knocked it down (KD) by siRNA in the mimic transfected SFbs. ADAMTS8 KD remarkably ceased the upregulation of COL14A1, COL5A1 and FN1, implying that regulation of these genes by miR-27b-3p may in part be ADAMTS8 dependent, while COL1A1 and THSB1 are independent of ADAMTS8. Transwell migration indicated a significant increase in the migration of miR-27b-3p transfected SFbs in comparison to control. In vivo studies using ISH showed that miR-27b-3p expression is prominent in the synovial lining and fibrocartilage of DMM-induced OA mice knee joints compared to sham control mice.

CONCLUSIONS: Our results thus far suggest that, in OA SFbs, miR-27b-3p is involved in the regulation of key ECM components, which seems to be in part via the modulation of ADAMTS8. We also found that miR-27b-3p is involved in the migration of OA SFbs: ongoing studies are confirming if this migratory role of miR-27b-3p is controlled by ADAMTS8 signalling. Our in vivo studies show that miR-27b-3p expression is elevated in the synovium during OA. To comprehensively investigate the endogenous role of miR-27b-3p in synovial pathology, we are intra-articularly injecting mouse knee joints with the in-vivo grade mimic of miR-27b-3p to determine any changes in the synovium upon miR-27b-3p induction in vivo.

T9.

The FIAT transcriptional repressor as a drug target for bone regeneration

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Activating transcription factor 4 (ATF4) is a member of the basic domain/leucine zipper family of transcription factors that can dimerize with other leucine zipper proteins. ATF4 plays a pivotal role in regulating osteoblast differentiation and function. Our laboratory has previously identified FIAT (Factor Inhibiting ATF4-mediated Transcription), also a leucine zippercontaining factor, as a binding partner that inhibits the transcriptional activity of ATF4. Transgenic mice overexpressing *Fiat* exhibit a low bone mass phenotype while a global *Fiat* knockout showed increases in cortical and trabecular bone thickness. Through these studies FIAT has emerged as a valid drug target for bone regeneration.

The goal of this study was to use a high throughput screen to identify compounds that disrupt the ATF4/FIAT interaction and thus find small molecules that could be used in the clinical setting to increase bone mass. We have generated a FIATzipper-GAL4-DNA Binding Domain fusion (DBD-FIAT 'bait') and an ATF4zipper-VP16 Activation Domain fusion (AD-ATF4 'target') to establish a mammalian two-hybrid assay. In this assay, the DBD-FIAT fusion binds the promoter of a secreted luciferase reporter construct. Interaction of the bait with the AD-ATF4 target through the leucine zipper interface reconstitutes a strong transcriptional activator and results in high levels of luciferase expression. Any compound that disrupted luciferase expression in the two-hybrid assay without influencing transcription mediated by the fused GAL4-VP16 control was considered a hit. After screening over 135,000 compounds and performing dose-response testing, 8 compounds were selected for additional screening to determine the tolerance, specificity and effects on osteoblast differentiation and mineralization. Preliminary data has defined the tolerance/survival of MC3T3-E1 osteoblastic cells upon treatment with the drugs, allowing for further investigation of the effects of the compounds on differentiation and mineralization. Three (3) compounds have a positive effect on differentiation and mineralization of MC3T3-E1 cells as measured by alkaline phosphatase and calcein staining, respectively. These preliminary data support the hypothesis that compound(s) blocking the interaction of FIAT with ATF4 would increase osteoblast activity and provide valuable information for further in-depth *in vitro* experiments and *in vivo* pilot studies.

T10.

Ghrelin and des-acyl ghrelin binding in cardiac tissue are altered with cardiovascular inflammation in Duchenne muscular dystrophy

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INTRODUCTION: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive, neuromuscular degenerative disorder that affects approximately 1 in every 3000 male births. It is characterized by progressive muscle degeneration that worsens with time and can be fatal at early stages. DMD is the result of the absence of the dystrophin protein leading to necrosis and inflammation in the skeletal and myocardial muscles. Eventually, dilated cardiomyopathy develops from microenvironmental deficiencies including ischemia, inflammation and fibrosis. Hence, the characterization of cardiovascular inflammation is critical to develop therapies for the cardiomyopathy of DMD. As noted, both cardiomyocytes and vascular endothelial cells produce the hormone ghrelin and its receptor, the growth hormone secretagogue receptor (GHSR). Thus, these two markers can be used as an indication of cardiovascular inflammation in DMD. Methods: We used a mouse model of DMD in which both dystrophin and utrophin have been deleted (mdx:utrn-/-). We have previously detected cardiomyopathy at 15-17 weeks of age in these mice. Here, we detected cardiomyopathy at 8-10 weeks at age in the same mice. In both littermate control and mdx:utrn-/- mice (n=3), myocardial tissues were assessed for GHSR using the fluorescent peptide analog Cy5-ghrelin (1-19). Des-acyl ghrelin binding was detected with Cy5-des-acyl ghrelin (1-19). To determine whether GHSR levels correlate with an inflammatory phenotype, levels of interleukin-6 (IL-6), F4-80(Macrophage marker), and the fatty acid transporter CD36 were quantified by immunofluorescence. Isolectin was used to detect vasculature. Masson's trichrome staining was used to detect fibrosis, and hematoxylin and eosin (H&E) staining was used to detect cardiac tissue degeneration. Fluorescence intensities were determined using NIS Elements AIR 5.02.00 software. Two-way ANOVA was used to compare the levels of GHSR between the DMD and aged-matched control groups. To correlate the level of inflammation with GHSR, linear regression analysis was run on the F4-80 vs. GHSR data and on IL-6 vs. GHSR data.

RESULTS: There were significant increases in levels of GHSR ($p<0.0001$), IL-6 ($p<0.0001$) and CD36 ($p<0.005$) in the myocardium of mdx:utrn-/- mice. There was strong positive correlation between GHSR and F4-80 ($p<0.0004$, r =0.966) and GHSR and IL-6 ($p<0.002$, r =0.926). Large amounts of collagen deposition as well as regions of necrosis were evident throughout the cardiac tissue, indicating severe fibrosis. CD36 correlated positively with fibrosis and necrosis ($p<0.02$, r=0.925). Interestingly, large cardiac vessels, but not microvessels, were strongly positive for Cy5-des-acyl ghrelin (1-19) in DMD mice ($p<0.02$).

CONCLUSION & SIGNIFICANCE: We report that GHSR associates with cardiac inflammation and that both CD36 and des-acyl ghrelin binding associate with vascular inflammation in mdx:utrn-/- mice. Des-acyl-ghrelin could be a marker of inflammation in large blood vessels in the heart. We propose that GHSR and des-acyl ghrelin are markers of cardiovascular inflammation in DMD.

Mechanisms of Matrix Remodeling and Disease

T11.

A circular RNA promotes tumor progression

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Intra-tumoral heterogeneity fosters tumor cell evolution and is an important consideration in both treating and studying cancer. Currently, mechanisms by which tumor cells propagate resistant sub-clones are not widely appreciated. Non-coding RNAs including circular RNA have been implicated in this process. We found that breast cancer cell invasiveness was positively correlated with circSka3 expression, through the formation of invadopodia via binding of Tks5 and integrin-beta1. Notably, we found that circ-Ska3 was complexed with Tks5 and integrin-beta1 in tumor-derived exosomes. This heterogeneously expressed circular RNA was further found to be transmitted via tumor-derived exosomes to potentiate the invasive potential of breast cancer cells expressing lower levels of circ-Ska3. Thus, we provide evidence that the transmission of circular RNAs in tumor-derived exosomes may allow for the maintenance of advantageous invasive subclones in breast cancer.

T12.

ATF6α/CHOP arm deficiency drives lung fibrosis via myeloid-derived macrophages

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BACKGROUND/OBJECTIVES: Pulmonary fibrosis is an irreversible fibrotic disease that is characterized by myofibroblasts accumulation that leads to a progressive increase in lung stiffness and a decline in function. Recent evidence suggests that alternatively activated macrophages (AAMs) are involved in the fibrotic processes, possibly via activating fibroblast to myofibroblast transition. Recently, we showed that full body knockout of C/EBP homologous protein (CHOP), a protein involved in the unfolded protein response (UPR)-associated apoptosis, enhanced the fibrotic response and this was associated with the accumulation of AAMs. However, it is not fully understood if these observations were specific to gain of function in the myeloid-derived cells or due to the activation of other cell types, and if targeting AAM would be sufficient to prevent progression of fibrosis. Therefore, we here investigated the specific role of AAMs in fibrotic lung disease. We hypothesized that selective inhibition of UPR-mediated apoptosis in the myeloid compartment will result in accumulation of AAMs and enhance the fibrotic response. To test our hypothesis, we have created a myeloid-specific knockout of ATF6α, known to be required for CHOP induction and cell apoptosis. Additionally, we have characterized macrophage accumulation and alternative activation in human fibrotic lung disease.

METHODS: 9-14 weeks ATF6α knockout within the myeloid lineage (ATF6αm-/-) and wildtype C57BL6/J (ATF6α+/+) female mice were intubated intratracheal with either low dose of bleomycin (0.04U) or sterile saline alone. After 7 and 21 days of bleomycin exposure, broncheoalveolar lavage fluid (BALF) was collected for cell differentials and macrophages from remaining right lungs were characterized by flow cytometry. Lung function was evaluated by flexiVent® assessment and histological examination was conducted using aSMA, Trichrome, and CD206 (AAM marker) staining. Furthermore, human lung tissues were obtained from a biobank for lung diseases and a tissue microarray (TMA) was generated. RNAScope *in situ* hybridization was performed on the human TMA to co-localize CD68 (macrophages marker), CD206 and either ATF6α or CHOP at the RNA level.

RESULTS: Myeloid lineage ATF6α deficient mice exhibited an increased fibrotic response when exposed to bleomycin, shown by histological examination including macrophage polarization (CD206), myofibroblast accumulation (αSMA) and Masson's Trichrome Blue staining as well as lung elastance ($p<0.05$). Flow cytometry assessments indicated that CD64+CD11c+ alveolar macrophages, CD206+ AAMs and MHCII+CD64+ activated macrophages were accumulated in the lungs of bleomycin-exposed ATF6αm-/-. Additionally, we have shown that ATF6α and CHOP genes were expressed in CD68+CD206+ AAMs found in IPF patients.

CONCLUSION: Overall, the data presented indicate that the selective depletion of ATF6α from the myeloid lineage increases macrophage accumulation and the fibrotic response in mice exposed to a low dose of bleomycin due to CHOP pathway deficiency. This suggests a protective role of ATF6α/CHOP arm and a profibrotic role of myeloid-derived cells in lung fibrogenesis.

T13.

Time-lapse microCT-based in vivo imaging reveals increased bone formation in mice with multiple myeloma bone disease

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INTRODUCTION: Multiple myeloma (MM) is an incurable plasma cell derived neoplasia. Patients develop devastating osteolytic lesions that lead to non-healing fractures and pain. We aimed to assess the adaptive cortical bone (re)modeling response to mechanical loading in a mouse model of MM. We hypothesized in vivo tibial loading would have an anabolic and anticatabolic effect in tumor-injected tibiae, rescuing bone loss.

METHODS: Ten-week-old female Balb/c mice underwent in vivo strain-matched dynamic compressive loading of the left tibia (5d/wk for 20 days, M-F; right tibia internal nonloaded control). The mice were injected 14 days prior to loading with either MOPC315.BM cells (mimic human MM) or PBS (left limb injected, right limb noninjected) or were not injected (n=7, left limb noninjected, right limb noninjected). Tumor- and PBS-injected mice were randomized into loaded (left limb loaded, right limb nonloaded) or nonloaded (left limb nonloaded, right limb nonloaded) groups (n=6-9/group). In vivo microCT was performed at day 13, 18, 23, 28 and 33 post injection. MicroCT images from day 13 and 33 were geometrically registered to monitor bone formation and resorption at the endosteal and periosteal tibial cortical metaphyseal surface: normalized newly mineralized and eroded bone volume (MV/BVday0-20, EV/BVday0-20), bone surface areas (MS/BSday0-20, ES/BSday0-20), and mineralized thickness and eroded depth (MTh day0-20, ED day0-20). An ANOVA assessed effects of treatment (tumor injected/PBS injected /noninjected) and loading (loaded/nonloaded) ($p \leq 0.05$). Fluorochrome-based histomorphometry was also performed. Tumor progression was monitored using bioluminescence imaging and enzyme-linked immunosorbent assay to detect MOPC315.BM specific immunoglobulin A (IgA) levels.

RESULTS: Loading increased periosteal MV/BV and MS/BS and decreased periosteal EV/BV and ES/BS compared to nonloaded limbs in all groups. Loading-induced increases in endosteal MV/BV was only observed in the tumor group. Endosteal MS/BS was diminished, while ES/BS was increased in tumor injected mice compared to PBS injected and noninjected groups. Loading-induced effects on the eroded surface and volume suggests fewer resorption sites at the periosteal surface. Injection, either tumor or PBS, altered periosteal and endosteal bone formation and resorption, suggesting a regional acceleratory phenomenon. Load induced increases in cortical bone mass were accompanied by decreased tumor burden as evidenced by MOPC315.BM specific IgA levels. Our data in young mice with MMBD indicate that mechanical loading not only rescues osteolytic bone loss but also has anti-myeloma effects. These data suggesting a combination of load-bearing exercise with bone resorption inhibitors in future clinical therapeutic strategies.

T14.

Fibulin-4 exerts a dual role in LTBP-4 mediated matrix assembly and function

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INTRODUCTION: Elastogenesis is a hierarchical process by which cells form functional elastic fibers, providing elasticity and the ability to regulate growth factor bioavailability in blood vessels, lung and skin, among other tissues. This process requires accessory proteins, including fibulin-4 and -5 and latent transforming growth factor binding protein (LTBP)-4. Mutations in these proteins cause a variety of heritable disorders resulting in deficient elastic fibers and function.

RESULTS: Our data demonstrate novel mechanisms in elastogenesis, focusing on the interaction and functional interdependence between fibulin-4 and LTBP-4 and its impact on matrix deposition and function. We show that LTBP-4 is not secreted in the expected extended structure based on its domain composition, but instead adopts a compact conformation. Interaction with fibulin-4 surprisingly induced a conformational switch from the compact to an elongated LTBP-4 structure. This conversion was only induced by fibulin-4 multimers associated with increased avidity for LTBP-4, fibulin-4 monomers were inactive. The fibulin-4-induced conformational change caused functional consequences in LTBP-4 in terms of binding to other elastogenic proteins, including fibronectin and fibrillin-1, and of self-assembly. A transient exposure of LTBP-4 with fibulin-4 was sufficient to stably induce conformational and functional changes, a stable complex was not required. These data define fibulin-4 as a molecular extracellular chaperone for LTBP-4, a novel paradigm in the field. The altered LTBP-4 conformation also promoted elastogenesis, but only in the presence of fibulin-4, which is required to escort tropoelastin onto the extended LTBP-4 molecule.

CONCLUSIONS: Altogether, this study provides a novel dual mechanism for fibulin-4 in i) inducing a stable conformational and functional change in LTBP-4, and ii) promoting deposition of tropoelastin onto the elongated LTBP-4. This novel fundamental finding is essential to understand the pathogenesis of diseases associated with mutations in elastogenic proteins.

T15.

Transglutaminase activity and enzyme expression is regulated by M-CSF and RANKL in osteoclasts

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Bone resorption is orchestrated by osteoclasts which are large multinucleated macrophage lineage cells capable of resorbing mineralized bone. Increased osteoclast activity causes bone loss, i.e., osteopenia. Osteoclast differentiation includes multiple steps; differentiation, migration, fusion which occur in cultures within 6 days upon stimulation of bone marrow macrophages (BMMs) with M-CSF and RANKL. Transglutaminases are a family of Ca2+ and thiol-dependent acyl transferases that catalyze covalent crosslinking, i.e., isopeptide formation between peptide-bound glutamines and amine groups such as lysine-residues. TG family consists of eight catalytically active enzymes, TG1-7 and Factor XIII-A which share 100% homology of their active site residues, yet different substrate specificities implying different functions. Recent studies have shown *in vitro* and *in vivo* that deletion of TG2 increases osteoclast numbers and deletion of FXIII-A decreases the process. We have shown that double null of TG2 and FXIII-A has dramatically increased resorption and that TG1 is also expressed during osteoclastogenesis. Furthermore, we have shown that five different TG inhibitors block osteoclast. These data demonstrate that TG activity is required for the process, but that a complex regulatory circuitry exists between the three enzymes. The aim of this study was to characterize in detail the total TG activity in osteoclastogenesis and the expression and activity of the individual enzymes to understand better the roles they might play in osteoclast differentiation. Osteoclasts were differentiated from BMMs with M-CSF and RANKL. Total activity was assessed in cells each day using 5- (biotinamido)pentylamine (bPA) and specific activities of TG1, TG2 and FXIII-A, was analyzed using specific biotinylated 'Hitomi-substrate peptides', bK5, bT26 and bF11. We report that TG-activity during osteoclastogenesis is highest on days 0-3, of differentiation and decrease at day 4 to essentially none. Examination of roles of M-CSF and RANKL in the modulation of activity showed that it was dramatically upregulated by M-CSF and downregulated by addition of RANKL in the cells. qRTPCR analysis of *Tgm1* expression showed significant, upregulation of its mRNA by M-CSF and with joint treatment of M-CSF and RANKL. M-CSF showed significant upregulation of *Tgm1* mRNA and activity as assessed with bK5 peptide, whereas RANKL downregulated both expression and activity. *Tgm2* analysis showed upregulation of its mRNA with M-CSF and RANKL treatment together. In contrast to *Tgm1*, *Tgm2* was significantly upregulated by RANKL at transcriptional level, but its activity as assessed by bT26 peptide, was downregulated. *F13a1* analyses showed high activity and mRNA in BMMs, but a significant and complete downregulation of its mRNA and activity by RANKL which strongly suggests that its involvement in macrophages only. Based on the results, we conclude that TG activity in osteoclasts arises from TG1 and TG2 whereas FXIIIA likely has no role in the differentiation.

T16.

The effect of pH on disc cells

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INTRODUCTION: Low back pain is one of the most prevalent medical conditions, affecting up to 84% of all people. Despite its widespread implications, the molecular cause of it has yet to be determined. Low back pain is caused by the degradation of the intervertebral discs—small, compressible discs that aid in the dispersion of weight between your vertebrae. Currently, the disease is thought to occur from a buildup of inflammatory molecules, either from trauma or over time with age. It is known that as the disc degenerates, the pH becomes more acidic, as low as 6.0 and in extreme cases, even lower. The role of toll-like receptors in the disc is becoming well studied; TLRs are known to be a major player in the progression of disc degeneration because of the upregulation of inflammatory cytokines resulting from TLR signaling, and their ability to recognize endogenous ligands, including extracellular matrix (ECM) fragments. It is not known if TLR signaling, and the resulting down-stream effects on gene expression, is altered by the pH alone.

AIM: Determine the effect of pH on gene expression of factors associated with disc degeneration and the effect of pH on TLR receptor expression and signaling.

HYPOTHESIS: We expect that acidic pH (<6.8) will increase inflammatory cytokine production.

Methods: We are fortunate to receive human lumbar spines through a partnership with Transplant Quebec, from which we isolate cells and store in our cell bank. It has been shown that acidic pH can decrease cell viability drastically after four days of culture, so a 48-hour culture was chosen. Human nucleus pulposus cells were cultured under three different conditions: pH 7.4, 6.8, and 6.2 with 250,000 cells/well in a 6-well plate. Pam2CSK4, a TLR 2/6 agonist, was used to treat cells for the 48 hours at a concentration of 1ug/mL. RNA was then extracted and PCR was performed to assess relative gene expression of IL-6, MMP3, TLR2, and NGF.

RESULTS: We found an increase in IL-6 gene expression at pH 6.8 and 6.2 with 2- and 3-fold respectively. We found a slight increase in MMP3 expression, and a slight down-regulation of TLR2 and NGF gene expression but no effect of pH on gene expression of, TLR2, and NGF after two days of culture. There was a strong induction of IL-6 and MMP3 after treatment with the TLR 2/ agonist. However, there was only a slight induction of TLR2 and a decrease in NGF expression across all pHs following TLR 2/6 activation.

SIGNIFICANCE: Since the pH in intervertebral discs is known to decrease as they become more degenerate, it is important to see if it alone can cause changes to catabolic factors. It is also important to see if pH affects cellular signaling after stimulation with an agonist, because there could be the potential for a synergistic effect.

T17.

How do fibronectin mutations cause "corner fracture" type spondylometaphyseal dysplasia?

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Fibronectin is a multimodular glycoprotein existing in two isoforms. The soluble form is known as the plasma fibronectin, produced and secreted by the liver. The insoluble cellular fibronectin forms fibers in the extracellular matrix (ECM). Cartilage development involves aggregation of undifferentiated mesenchyme into a condensed mass of cells. The cells then proceed to differentiate into chondrocytes, which facilitate cartilage development through ECM protein production and secretion. During mesenchymal condensation, fibronectin is abundantly expressed, promoting migration of mesenchymal cells as well as their differentiation. Thus, impaired production or secretion of fibronectin may affect chondrocyte differentiation and development of cartilage. Spondylometaphyseal dysplasias (SMD) are a heterogeneous group of conditions affecting the spine and growth plates. One of these conditions include the corner-fracture type SMD, a rare skeletal dysplasia with short stature and numerous other skeletal phenotypes. We previously discovered several heterozygous mutations in the fibronectin gene (*FN1*), upon whole exome sequencing of 12 suspected corner fracture SMD patients. These mutations are dispersed across the N-terminal type -1-module domains, and include p.Cys87Phe, p.Tyr240Asp and P.Cys260Gly, among others.

To determine the consequences of these missense mutations in fibronectin production and secretion, we produced recombinant, secreted 70kDa N-terminal fragment (rF70K) as well as a full-length recombinant fibronectin (rFN). We also developed mutant constructs by introducing the SMD mutations p.Cys87Phe, p.Tyr240Asp, and p.Cys260Gly into rF70K and rFN. The full-length wild-type rFN as well as the mutants were transfected into the chondrogenic cell line ATDC5. Wild type and mutant rFNs showed similar levels of expression on the mRNA level when analysed by RT-PCR. Protein expression analysis using Western blotting and immunofluorescence staining revealed synthesis of both transfected wild type and mutant rFN proteins. ATDC5 cells secreted wild type rFN into the culture medium. However, the transfected cells secreted very little or no mutant rFN into the culture medium. Immunofluorescence analysis revealed intracellular retention of these mutant proteins. We are now analysing the consequences of these fibronectin mutations on chondrocyte differentiation, using the transfected ATDC5 cells.

In summary, mutations in the fibronectin gene, found in corner - fracture type SMD patients do not alter fibronectin production by cells, but impair secretion of the mutant proteins. The results indicate the involvement of these fibronectin mutations in dysregulating chondrocyte differentiation and impairing proper cartilage development, leading to spondylometaphyseal dysplasia.

T18.

Further characterization of the 67 kDa laminin receptor (67LR) in colorectal cancer cells

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The 67LR (67 kDa laminin receptor) was the first non-integrin cell surface receptor for laminin to be discovered in the 1980's. Initially, the 67LR was found as a cytoplasmic precursor of 37 kDa named RPSA (40S ribosomal protein SA) associated with the small ribosomal subunit. In human cells, RPSA allows the formation of the polyribosome complex and plays a key role in the initiation of translation. The mechanism by which the ribosomal protein becomes the 67LR membrane receptor is still unclear. It is presumed that the process involves post-translational modifications combined with homo or heterodimerization with non-associated ribosomal proteins. It has been shown that 67LR regulates adhesion and proliferation of normal human intestinal epithelial cells. Interestingly, overexpression of 67LR is correlated with aggressiveness and a poor prognosis in a wide variety of cancers. The aim of this study was to confirm the overexpression of the 67LR in the membrane of colorectal cancer cells and identify its homo or heterodimerization partners. To confirm the presence of 67LR at the membrane of Caco-2 cells we used a cellular fractionation protocol combined with ultracentrifugation and detergent treatment to separate ribosome-containing fractions from the membranes and isolate the membrane associated 67LR. Following analysis by western blotting, immunoreactive 67LR protein was found in the soluble fraction after ultracentrifugation at 210,000xg while a 37 kDa form was detected in the insoluble counterpart which was solubilized after treatments with detergent, suggesting that the 37 kDa form is associated with the membrane. These results suggest the existence of a soluble cytoplasmic dimer of 67kDa, a precursor to the formation of the 37 kDa membrane receptor. Mass spectrophotometry analysis of these fraction are ongoing in order to get an indication of the identity of the soluble dimeric partner and to understand the mechanism of the involvement of 67LR in colorectal cancer.

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T19.

Regulation of adipogenesis and adipose tissue homeostasis by fibrillin-1

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BACKGROUND: Fibrillins are large extracellular glycoproteins ubiquitously expressed in elastic and non-elastic tissues throughout the body. Marfan syndrome (MFS) is a heritable disorder caused by mutations in fibrillin-1. Lipodystrophy as well as obesity are relatively common in adults with MFS, and both are known to increase the risk of cardiovascular complications. Moreover, fibrillin-1 expression in obese women correlates with obesity and increased adipocyte size.

AIM: The aim of this study is to understand the role of fibrillin-1 in adipocyte differentiation and fat metabolism using MFS mouse models.

METHODS: *In vitro*, mouse bone marrow-derived mesenchymal cells (MSCs) were cultured for 10 days with an adipogenic cocktail (insulin, dexamethasone, isobutylmethylxanthine, and indomethacin) with or without recombinantly produced fibrillin-1 N-terminal (rFBN1-N) and C-terminal (rFBN1-C) halves. Adipocytes were identified with the lipophilic dye Oil Red O, and adipocyte count was evaluated. Surface plasmon resonance spectroscopy (SPR) was used to study the interaction of fibrillin-1 with insulin. For *in vivo* experiments, we analyzed two MFS mouse models, *Fbn1*mgR/mgR and *Fbn1*C1039G/+. *Fbn1*mgR/mgR are hypomorphic mice that produce reduced amounts (25%) of normal fibrillin-1. *Fbn1*C1039G/+ carry a missense mutation in fibrillin-1 (C1039G). MSCs from *Fbn1*mgR/mgR, *Fbn*C1039G/+ and wildtype mice were cultured with adipogenic cocktail and adipocyte count was determined with Oil Red staining. We measured the weight of white adipose tissue (WAT), brown adipose tissue (BAT) and serum insulin concentrations at 16 weeks old *Fbn1*mgR/mgR and 35 weeks old *Fbn1*C1039G/+ mice and their respective wildtype littermates controls (LC). Serum insulin concentrations were analyzed by ELISA.

RESULTS: We found that rFBN1-C significantly reduced adipocyte differentiation *in vitro* while rFBN1-N did not influence the adipocyte differentiation. While maximum inhibition of adipogenesis was achieved when rFBN1-C was present for the entire duration of the experiment, a significant decrease in adipocyte numbers was evident when rFBN1-C was present during the first 3 days of differentiation. SPR analysis showed that insulin can directly interact with fibrillin-1. The dissociation constant (Kd) indicated stronger binding of rFBN1-C (Kd=143 nM) to insulin compared to rFBN1-N (Kd=359 nM). An increased number of adipocytes was observed when MSCs from *Fbn1*mgR/mgR mice were cultured in adipogenic medium compared to LC, while no significant difference was observed between adipogenic differentiation of MSCs from *Fbn*C1039G/+ mice and their LC. Male *Fbn1*mgR/mgR mice displayed a significantly higher weight of BAT and WAT compared to the LC. Male *Fbn*C1039G/+ mice showed a trend in increased weight of BAT, but not WAT, compared to LC. The whole-body weight was not significantly different between *Fbn1*mgR/mgR and *Fbn1*C1039G/+ mice and their LC. Hyperinsulinemia was observed in *Fbn1*mgR/mgR male mice compared to LC. Adipose tissue indices in female *Fbn1*mgR/mgR and *Fbn1*C1039G/+ mice were similar to their LC.

CONCLUSION: Fibrillin-1 haploinsufficiency or a fibrillin-1 missense mutation in male mice caused an increase in fat mass. Moreover, C-terminal half of fibrillin-1 interfered with adipogenesis likely by sequestering insulin from the culture medium. Thus, our study indicates a novel mechanism of action by which fibrillin-1 reduction or mutation may affect whole body adipose tissue homeostasis.

T20.

Isomerism affects polyaspartate inhibition of calcium phosphate crystallization

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Proteins which regulate collagen mineralization contain substantial amounts of aspartate and glutamate, leading to the use of polyaspartate and polyglutamate as analogues of such mineralizing proteins for *in vitro* models. Despite the structural similarity of aspartate and glutamate, their homopolymers are reported to have substantially different effects on calcium phosphate mineralization; polyaspartate is an effective inhibitor of hydroxyapatite formation in solution, while polyglutamate is more enigmatic, exhibiting both nucleation and inhibitory capabilities depending on the experimental conditions. We hypothesize that the difference between these peptides is at least partially due to optical and structural isomerism observed in many samples of polyaspartate, the result of which is disruption of secondary structure and enhanced flexibility. We use nuclear magnetic resonance spectroscopy and polarimetry to characterize the extent of structural and optical isomerism in samples of polyaspartate and polyglutamate, and investigate secondary structure of these peptides using circular dichroism spectroscopy. These data are correlated with inhibition of hydroxyapatite formation using a turbidity assay to show that structural and optical isomerism enhances the inhibitory effect of polyaspartate and polyglutamate. This research has the potential to enhance our understanding of the roles of glutamate and aspartate repeats in mineralization-associated proteins, and to assist in design of synthetic mineralization-directing polymers.

T21.

Kindlin-2 mediates mechanical activation of cardiac myofibroblasts

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BACKGROUND: Upon tissue injury, fibroblasts are activated into myofibroblasts, which secrete and contract extracellular matrix (ECM) into scar tissue to repair the defect; excessive scarring is called fibrosis. In the heart, fibrotic scar forming in response to cardiac infarct or chronic overload reduces cardiac muscle distensibility and pumping function and fosters arrythmia by changing electrical signal transmission. Better understanding of the molecular mechanisms that control myofibroblast activation is essential to treat and, ideally, prevent development of fibrosis. Transduction of mechanical signals from the ECM is at sites of focal adhesions key in controlling myofibroblast and activity, and activation (characterized by neo-expression of the contractile protein marker α-smooth muscle actin (α-SMA)). We identify the focal adhesion protein kindlin-2 as novel player in this process. Kindlins are FERM domain-containing adaptor proteins, bind to and activate integrins and regulate integrin-mediated cell-ECM interaction.

HYPOTHESIS: Kindlin-2 regulates mechanical activation of cardiac myofibroblasts.

Objective: To reveal a novel molecular mechanism through which kindlin-2 controls expression of α-SMA, the molecular marker and contraction-promoting in myofibroblasts.

METHODS: Primary human cardiac fibroblasts and rat embryonic fibroblasts were cultured on silicone substrates with stiffness corresponding to that of healthy heart (3 kPa), heart tissue under remodeling (26 kPa), or fibrotic heart (65 kPa), and on non-physiologically stiff tissue culture plastic. Fibroblasts were further subjected to active mechanical stimuli, such as tensile forces applied using magnetic microparticles, cell staining on silicone membranes, and the cell contraction agonist PAR1 (TFLLRN). Cell contraction as an indicator of myofibroblast activity was measured on silicone substrates that "wrinkle" upon cell contraction. Kindlin-2 expression and localization were analyzed using GFP-tagged constructs in live videomicroscopy, immunofluorescence staining, and western blotting of different cell fractions. Real-time RT-PCR and luciferase expression under α-SMA promoter control were used to assess the effect of kindlin-2 on α-SMA transcription.

RESULTS: Kindlin-2 was co-upregulated with α-SMA in human cardiac fibroblasts upon growth on fibrosis-stiff culture substrates. Stressing fibroblasts using magnetic microbeads, stretchable silicone membranes, and cell contraction agonists all resulted in kindlin-2 translocation to the nucleus. Overexpression of full-length kindlin-2 but not of kindlin-2 missing a putative nuclear localization sequence resulted in increased α-SMA promoter activity. Downregulation of kindlin-2 with siRNA lead to decreased expression of α-SMA and reduced myofibroblast contractility. These effects of kindlin-2 knockdown were prevented by overexpressing full-length kindlin-2 but not kindlin-2 missing the nuclear translocation sequence. The effect of kindlin-2 on α-SMA transcription was mediated via CC(A/T)-rich GG(CArG) box elements in the α-SMA promoter as revealed using reporter constructs missing these elements.

CONCLUSION: Kindlin-2 translocates to the nucleus in response to the mechanical transduction and directly controls myofibroblast activation by regulating α-SMA transcription. Because kindlin-2 acts as a mechanosensor, it can be a potential target to interfere with myofibroblast activation in tissue fibrosis.

Tissue Engineering/Repair/Regeneration

T22.

Highly porous elastomer scaffolds for ligament and cartilage tissue engineering

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BACKGROUND & OBJECTIVE: Ligament and cartilage damage often occurs as a result of sports-related injuries. Poor alignment of the joint, excessive weight, excessive activity, overuse or injury can cause further damage to these tissues. As avascular tissues, self-repair of cartilage and ligament is intrinsically limited, so intervention is required to recover the tissue structure. Mechanically, cartilage resists compressive forces, due to the high osmotic pressure that results from water interacting with negatively charged proteoglycans. Contrarily, ligament tissue exhibits strong properties when in tension; ligaments are subjected to a variety of load conditions that affect their mechanics. With proper scaffold design, the viscoelastic nature of these tissues may be replicated with LAY-FOMM; a thermoplastic polyurethane (TPU) co-polymer with polyvinyl alcohol (PVA). LAYFOMM scaffolds are produced using additive manufacturing, or 3D printing. These scaffolds themselves are nanoporous, with each scaffold incorporating three micro/macro-pores to allow for the deposition of ligament and chondrocyte cells and the formation of collagen-based matrices. By supplementing these scaffolds using 10% FBS, chondrogenic control and chondrogenic media, their ability to promote deposition and regeneration of collagen-based ligament/chondrocyte matrices can be investigated.

METHODS: Using fused-deposition modelling, these scaffolds are produced. All scaffolds were printed using a Flashforge Creator Pro, with a 0.3mm nozzle at a print temperature of 220°C, 50°C bed temperature, at 18mm.s-1. After printing, they were washed with distilled water to remove excess PVA, leaving a highly porous, flexible structure. The scaffolds were then washed with 70% ethanol for sterilization. Ligament and chondrocyte cells were held in culture for 21 days (high glucose DMEM, supplemented with 10% FBS, and 1% penicillin streptomycin), whilst changing media every 2-3 days. Following culture, the cells were counted, and seeded in the scaffolds in a 24-well plate, with 400,000 cells on each scaffold. Samples were prepared for each cell type (ligament and chondrocyte) and supplemented with: 1) 10% FBS media, 2) chondrogenic control media (serum-free), 3) chondrogenic media (serum-free, with TGF-β1). Following 21 days of culture, cell proliferation, viability (live/dead assay), western blot, and qPCR were performed.

RESULTS: The addition of serum (10% FBS) showed increased proliferation of chondrocyte and ligament cells. Deposition of ligament/chondrocyte extracellular matrix was visible in the pores of these scaffolds. Preliminary results show the expression of genes specific to chondrocytes and ligament-derived fibrocytes. Specifically, using qPCR, it was shown that cells from ligaments expressed higher levels of scleraxis and collagen type VI. Live/dead assay will further demonstrate the viability of ligament and chondrocyte cells, along with the viability of cells within the collagen-meshes. The proteins of interest for western blot of chondrocyte tissue are collagen II and aggrecan, while the protein of interest for ligament tissue is collagen I.

CONCLUSIONS: The use of LAYFOMM scaffolds can lead to the deposition of collagen-rich matrices. The anticipated results include the production of ligament and chondrocyte extracellular matrix, which may in-turn provide a novel treatment for tissue damage caused by sports-related injuries.

T23.

Design of a biaxial mechanobioreactor for engineering mechanically anisotropic connective tissue sheets

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INTRODUCTION: Tissue engineering human, anisotropic connective tissues such as skin, tendon, ligament, annulus fibrosis, and heart valves that biomimic their healthy native counterparts in function and biomechanical properties remains a challenge. Mechanical stimulation of engineered connective tissue (ECT) constructs is one paradigm to produce a biomimicked extracellular matrix. Unfortunately, there is a limited understanding of *in vitro* mechanical stimulation protocols. Bioreactors that apply cyclic biaxial tensile strain to anisotropic ECT constructs often lack the ability to modulate strain amplitude in each direction of application. In turn, they are unable to tune the anisotropic biomechanical properties in the ECT construct. There exists devices capable of uncoupled, planar biaxial stretch for small cell straining applications but they are not designed to uniformly strain larger ECTs (here up to ~ 60 mm). As such, we aim to design and optimize a novel bioreactor to biaxially stretch ECT sheets in a planar configuration.

METHODS: Finite element models were created in ANSYS 14.0 to: i) design a novel configuration that maximizes biaxial strain uniformity on ECT sheets; and ii) investigate strain patterns generated due to material and geometric anisotropy. To evaluate strain uniformity, a region was classified as uniform if the X and Y direction strain magnitudes were within 10% of the strain magnitudes at the center (midpoint) of the specimen. Configurations were compared to the trampoline setup, the literature gold standard for maximum strain uniformity. To evaluate strain patterns, midpoint strains were measured for different side lengths (from 20 mm to 60 mm), side length ratios ($1 \leq LX/LY \leq 3$), Young's moduli (1.2 MPa to 16 MPa) and material anisotropy ($1 \leq EX/EY \leq 13.3$). Solidworks was used to model prototypes that were then built in-house.

RESULTS: A novel "capstan+cruciform" configuration was determined to improve and maximize strain uniformity by 39.5% over the trampoline setup. The "capstan+cruciform" setup is designed to mount specimens without puncturing them and to maintain tension in corner regions, which the trampoline setup does not do. For strain pattern evaluations, the directional midpoint strain ratios ($\varepsilon x/\varepsilon y$) were found to be coupled to the specimen's Young's modulus anisotropy (EX/EY) and the side length ratios (LX/LY). Increasing the Young's modulus anisotropy led to a decrease in $\varepsilon x/\varepsilon y$ (EX/EY = 1, $\varepsilon x/\varepsilon y = 1$; EX/EY = 13.3, $\varepsilon x/\varepsilon y = 0.67$). Conversely, increasing side length ratios increased $\varepsilon x/\varepsilon y$ (LX/LY = 1, $\varepsilon x/\varepsilon y = 1$; LX/LY = 3, $\varepsilon x/\varepsilon y = 48.9$). Both the individual side lengths and Young's modulus had minimal effect on $\varepsilon x/\varepsilon y$.

CONCLUSION: We have designed a novel biaxial strain configuration that maximizes the applied strain uniformity for anisotropic ECT constructs inside a bioreactor. We demonstrated that strain magnitudes are coupled with the specimen's material and geometric anisotropy, parameters that are expected to evolve throughout culture. Since tissue constructs experience creep from cyclic straining and material properties evolve throughout culture, these results emphasize the need for directional strain modulation to ensure consistency in the strain applied to the cells of ECTs.

T24.

Development of a 3D microenvironment model for human bone metastases

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INTRODUCTION: The bone is a dynamic tissue that is constantly broken down and repaired through a balance between osteoclasts and osteoblast activity. However, when various cancers invade the bone, they can disrupt the physiological balance leading to various deleterious changes in bone structure and function. At the moment, radiotherapy, chemotherapy and surgical resection are the main clinical approaches to bone metastases. Studying cancer cells molecularly in vitro relies on 2D monolayer cultures which in no way represents the physiological tissue microenvironment in vivo. Therefore, preclinical and translational cancer research trends are moving toward organoid studies and 3D biomimetic models to provide more clinically relevance in all aspects. In this case, 3D bioprinting has become an important tool in developing 3D biomimetic in vitro models. Here, we set out to generate a bioink for bioprinting, consisting of alginate, gelatin, nano-crystal hydroxyapatite loaded with primary human osteoblasts to produce a robust 3D bone-like microenvironment in order to study human bone metastasis.

METHODS: A previously reported 3D hydrogel (3% alginate; 7% gelatin) model for cancer cell-migration was modified to incorporate nano-crystal hydroxyapatite, primary human osteoblasts and primary human bone marrow derived stromal cells (2 x 106 cells/mL). Primary osteoblasts were isolated from cadaveric vertebral bodies of organ donors, and bone marrow MSCs are purchased from Rooster Bio. The constructs were firsthand-cast and cultured for 28 days in either normal growth medium (DMEM) or osteogenic medium (OM) with and without 0.5 mg/mL hydroxyapatite (HA). Live/Dead assays will be performed to quantify viability at 28 days of culture. Fixed samples will be stained with Alizarin red for calcified matrix deposition, and hematoxylin and eosin to observe cells within the matrix. A second set was bioprinted using a tissue scribe and western blot will elucidate presence of bone matrix and osteocytes by probing for osteopontin and sclerostin. Finally, GFP labled MDA-MB-231 cells were co plotted with the human osteoblasts and cultured over 7 days

RESULTS: Live/Dead analysis revealed strong primary human osteoblast viability in all conditions after 28 days of culture: 91.3 ± 3.18 % for DMEM/HA+, 92.5 ± 2.5 % for DMEM/HA-, 88.6 ± 0.38 % for OM/HA+ and 85.9 ± 6.2 % for OM/HA-. Primary human MSCs showed approximately 85.1, 69.4, 87.8 and 90% viability in the same four conditions. Alizarin red staining showed that cells grown in DMEM without HA had the least amount of bone mineralized matrix. The combination found to have the most amount of bone mineralized matrix was OM/HA+. Western blot analysis shows presence of osteopontin and sclerostin across MSCs samples. Preliminary results of tumor cells co-cultures, shows cell growth and migration to periphery potentially providing a model for drug screening.

CONCLUSION: This work will allow better understanding of effect of the drugs on non-tumor cells and tumor cells in a more clinically relevant and physiological manner. The model will have broad implications for screening other pathological tissue and cell types as well as establish novel bone tissue engineering avenues.

T25.

Bone matrix formation by human mesenchymal stem cells on three-dimensional-printed polymer scaffolds

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BACKGROUND: The constantly rising numbers of sports-related injuries, cancer resections, congenital diseases, trauma, and spine surgery generate a high demand for bone grafting. The current gold standard for repair is the autologous bone graft. However, there is a limited quantity and associated donor site morbidity. Therefore, there is a need for alternative bone substitutes, which justifies the increased number of tissue engineering studies including the use of strong and inexpensive three-dimensional (3D)-printed scaffolds for bone regeneration. Several off-the-shelf materials are available for low-cost fused deposition 3D printing such as polylactic acid (PLA) and polycaprolactone (PCL). These polymers can easily be made in composites to include other factors such as minerals. In this study, we assessed three materials - PLA, Lactoprene 100M and Lactoprene 7415 with and without mineral additives of beta-tricalcium phosphate (β-TCP) - for their osteoinductive properties.

METHODS: Porous scaffolds (10x10x4mm, pore size 750μm) of PLA, and two novel materials Lactoprene 100M and 7415 supplemented with and without β-TCP were 3D-printed using a FlashForge Creator Pro desktop 3D printer. Their stiffness and hydrophilicity were assessed by unconfined compression and contact angle tests. Five hundred thousand human bonemarrow-derived mesenchymal stem cells (hMSCs) were seeded on the scaffolds and cultured for 21 days in standard and osteogenic cell culture medium respectively. Cell proliferation was assessed by Hoechst test for total DNA concentrations. Extracellular matrix (ECM) generation was evaluated by scanning electron microscope (SEM) and the calcified matrix was visualized and quantified by Alizarin red staining. Furthermore, we assessed osteogenic markers by Western blot for the evidence of bone matrix proteins.

RESULTS: 3D-printed scaffolds of all materials were successfully fabricated using a low-cost 3D printer. There was no significant difference in stiffness between PLA and 100M/100M+β-TCP. However, 7415/7415+β-TCP are ~69% weaker ($P<0.0001$) compared to PLA and 100M(+β-TCP). All scaffolds showed hydrophilic material properties. 100M/100M+β-TCP are ~20% more hydrophilic ($P<0.05$) than 7415/7415+β-TCP, and may be more biocompatible for tissue fusion in future implantation test. However, the numbers of cells adhering were ~71% and ~77% lower in 100M compared to 7415 ($P<0.01$)/7415+β-TCP ($P<0.001$), and ~53% and ~63% in 100M+β-TCP compared to 7415 ($P<0.05$)/7415+β-TCP ($P<0.001$). All materials supported cell proliferation and maintained cell numbers. 100M+β-TCP maintained ~12% more cells ($P<0.05$) than 7415+β-TCP in osteogenic condition. Though having ~63% less number ($P<0.001$) of cells at the beginning, 100M+β-TCP promoted ~60% and ~57% more calcified ECM formation than 7415+β-TCP in both standard ($P<0.0001$) and osteogenic ($P<0.0001$) conditions.

CONCLUSION: The current study focused on evaluating two innovative materials for bone tissue engineering and compared them to standard PLA. Our results highlight an improved osteogenesis and mineral deposition on materials supplemented with β-TCP. Our data also demonstrated superior properties of 100M+β-TCP, in its mechanical strength, hydrophilicity, and its significant promotion of cell proliferation and calcified extracellular matrix deposition. Further tests will evaluate bone matrix formation and biocompatibility *in vivo* and carry forward our goal of developing novel materials for bone regeneration.

T26.

Characterizing the roles of Wilms' tumor 1 variants in Dupuytren's disease development

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BACKGROUND: Dupuytren's Disease (DD) is a common and benign fibrosis of the palmar fascia, a sub-dermal connective tissue that extends from the base of the palm into the fingers. This fibrosis is characterized by excessive secretion and contraction of collagens and other extracellular matrix molecules, causing permanent and debilitating palmar-digital contractures. Available treatment options are associated with very high disease recurrence rates and this condition is currently considered incurable. Like many fibroses, chronic exposure to pro-inflammatory cytokines has been associated with DD development. Previous research in our laboratory has identified increased levels of *WT1* mRNA transcripts, encoding Wilms' Tumor 1 (WT1) proteins, in primary fibroblasts derived from fibrotic DD tissue compared to syngeneic fibroblasts derived from adjacent, visibly non-fibrotic palmar fascia. *WT1* is a context-dependent oncogene or tumor suppressor gene in leukemias, renal and other tumors where WT1 functions as a transcription factor and an RNA splicing factor. *WT1* is comprised of 10 exons with more than 30 alternatively spliced variants reported in various cancers. While there are many well-characterized molecular similarities between cancers and benign fibroses, the roles of *WT1* transcripts in palmar fascia repair and in the development of DD are currently unknown.

HYPOTHESIS: We hypothesize that a transient increase in *WT1* transcript expression is a normal, previously unrecognized component of the inflammation stage of palmar fascia repair, and that abnormally sustained expression of novel *WT1* transcripts promote DD development.

METHODS: *WT1* mRNA transcript variants were detected using Rapid Amplification of cDNA ends (RACE) and RT-PCR amplicon sequencing. Adenoviral vector transduction was used to express fibrosis-associated *WT1* transcript variants and to perform gain-of-function studies in fibroblasts derived from visibly non-fibrotic palmar fascia. Gene expression analyses were performed using qPCR.

RESULTS: Several different *WT1* mRNA variants were expressed in DD cells including the four major alternatively spliced variants previously reported in leukemias, a truncated variant transcribed from within intron 1 (*sWT1*) and a N-terminally extended transcript (*aWT1*). PF cells transduced with adenovirus expressing the *sWT1* variant expressed by DD cells exhibited increased *COL1A1* and *COL3A1* expression levels relative to controls.

CONCLUSIONS: We have identified the expression of two novel *WT1* transcripts (*sWT1* and *aWT1*), both previously reported to be pro-oncogenic in leukemia and breast cancer, in primary myofibroblasts derived from this benign connective tissue fibrosis. Our findings indicate that constitutive expression of the *sWT1* variant in "pre-fibrotic" myofibroblasts enhances collagen gene expression. To our knowledge, roles for these *WT1* transcripts have not been studied in the context of myofibroblast development and function in any disease. Future studies will include analyses to detect *WT1* transcripts that are induced by pro-inflammatory cytokine stimuli, and loss-of-function analyses using CRISPR/CAS9 to assess the effects of deleting these and other *WT1* transcripts in DD myofibroblasts. We anticipate that ongoing studies of *WT1* transcript expression will identify novel therapeutic targets to prevent DD progression and recurrence.

T27.

Role of endothelial ephrin-B2 signaling in pulmonary fibrosis

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INTRODUCTION: Fibrosis of the lungs and skin is a prominent feature of many diseases and mechanisms to explain the cause or development of fibrosis are unclear. We have previously identified the ephrin-B2 ligand as a novel mediator of skin and lung fibrosis (1). We showed that the conditional knockout (KO) of Efnb2 in collagen I expressing cells, including fibroblasts, partially protects mice against bleomycin-induced lung and skin fibrosis. This suggests that other cells, potentially endothelial cells through their interaction with fibroblasts, could contribute to tissue fibrosis through ephrin-B2 signaling. Therefore, this study aims to identify the contribution of endothelial-derived ephrin-B2 in the development of tissue fibrosis.

METHODS: With tamoxifen-induced Tie2-Cre genetic KO model on C57BL/6J background, we generated conditional Efnb2 KO mice. 4 week old mice underwent tamoxifen treatment (1mg/day; 5 days) or corn oil as control, and then treated with bleomycin sulphate (1.2 U/kg) at 6 weeks of age. Pulmonary fibrosis was assessed through histology 14 days post-treatment. Quantification of Gomori's One-Step Trichome was performed (50 fields at 20X); images were processed with ImageJ Color Deconvolution and quantified with Threshold Calculation. Percent of lung collagen was calculated using total lung tissue area as baseline.

RESULTS: The successful generation of Efnb2 KO mice post tamoxifen treatment was confirmed via genotyping for exon 1 deletion product. Through histomorphometric assessment, we found that endothelial Efnb2 KO exhibited significant protection against bleomycin-induced pulmonary fibrosis.

CONCLUSIONS: Our results thus far suggest that endothelial-derived ephrin-B2 may play a key role in either development or progression of pulmonary fibrosis.

1. Lagares et al. (2017) Adam10-mediated ephrin-B2 shedding promotes myofibroblast activation and organ fibrosis. Nature Medicine. 23, 1499

T28.

Integration Analysis of Matricellular Protein Expression Signatures in Mechanistically Different Mouse Models of Kidney Injury

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INTRODUCTION: Chronic kidney disease (CKD) is a major health concern affecting up to 10% of the global population. Fibrosis is a maladaptive condition of chronic repair associated with a majority of CKDs. It is characterized by aberrantly excessive accumulation and processing of stiff extracellular matrix (ECM) materials, which progressively replaces the flexible parenchymal tissue to the point of organ failure, and eventually death. Over the last 20 years, Matricellular proteins (MPs) have emerged as essential regulators to these ECM events, which consequently give them a fundamental role in tissue repair. They also exhibit contextual expression, typically induced transiently during a normal repair process. However, their persistent expression as in chronic injuries can shift their function from being restorative to fibrotic.

OBJECTIVE: To identify and analyze significant MP expressions in two mechanistically distinct models of kidney injury during the transition from an Acute-to-Fibrotic repair process.

METHODS: Using published RNA-seq data from two kidney injury models of different etiologies, Folic Acid (FA) and Unilateral Ureteral Obstruction (UUO), we searched for 29 members of 5 common MP families (CCN, SPARC, TSP, SIBLING, TN) and analyzed their temporal RNA expression patterns during the acute injury phase and identified those that were sustained into chronic pathologies. STRING analysis coupled with GO enrichment of differentially expressed genes uncovered MP networks and predicted biological functions in the context of kidney fibrosis. Validation of specific MPs was done by Western blotting using kidney lysates of C57BL/6J mice subjected to FA treatment or UUO surgery and correlated with kidney injury, as assessed by fibronectin expression and kidney functional assays.

RESULTS: From our analysis, we present the expression profile of 29 members from 5 MP families, most of which, to the best of our knowledge, have not been previously presented over the progression of UUO and FA injury, and in some cases have never been studied in kidney fibrosis nor fibrosis in general. The Fibrotic phase (>7day injury) experienced a larger number of differentiated MP genes than the Acute phase (<7day injury). Within a Molecular Function network, top annotation clusters that similarly appeared for FA and UUO were binding to ECM proteins heparin, fibronectin, integrin and collagen. We found that such interactions were relatable to their top Biological Processes, such as adhesion, ECM organization, development and proliferation, all of which are typically considered in repair processes. The RNA-Seq data from both injury models of UUO and FA were validated for their protein expression by selecting CCN2 and OPN as representative early MPs and SMOC2 and Fstl1 as representative late MPs. Interestingly, all candidate MPs mirrored the severity of the injury, where MP expression followed the typical injury regressive and progressive patterns of FA and UUO, respectively. Conclusion: Understanding the mechanisms of MPs will provide important insight into the injury and repair processes of the kidney, and may validate MPs as therapeutic targets for managing kidney repair before fibrotic onset for which there is currently no approved treatment.

T29.

Myofibroblasts derived from palmar fascia fibrosis elicit changes in cytokine gene expression inTHP-1 monocytes

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BACKGROUND: Palmar fascia fibrosis, aka Dupuytren's disease (DD), is characterized by myofibroblast-driven palmar-digital contractures of the underlying connective tissue. Chronic or other abnormal interactions between myofibroblasts and immune cells during the inflammation stage of palmar fascia repair have been implicated in promoting fibrosis development. While previous studies have mostly concentrated on how macrophages modify myofibroblast gene expression and behaviors to promote fibrosis, less is known about the ability of myofibroblasts to modify cytokine gene expression and secretion by monocytes and/or macrophages.

HYPOTHESIS: We hypothesize that myofibroblasts derived from fibrotic palmar fascia (DD cells) elicit signals that induce abnormal cytokine gene expression and secretion profiles in monocytes/macrophages, thereby promoting fibrosis development.

MATERIALS AND METHODS: THP-1 cells, an immortalized undifferentiated human monocyte cell line, were co-cultured with primary palmar fascia myofibroblasts derived from DD tissues (DD cells) or from normal palmar fascia (CT cells). DD or CT cells (1.2 x 105cells/well) were cultured in stressed Fibroblast Populated Collagen Lattices (FPCLs) for 72 hrs. to promote myofibroblast development, after which THP-1 cells (1.0 x 105cells) were layered onto the FPCLs. THP-1 cells were also cultured onto cell-free collagen lattices, with or without LPS (1mg/ml), as positive or negative controls respectively. After 6 days, non-adherent THP-1 cells were isolated and processed for RNA extraction, reverse transcription and semi-quantitative PCR analyses to assess *CCL2, TNF, IL1B, IL6, IL10, FN* and *TGFB* expression levels.

RESULTS: LPS treatments increased *IL1B, IL6, IL10* and *FN* expression in THP-1 cells relative to untreated cells, while little or no effects on *TNF*, *CCL2* and *TGFB* expression were evident. Our preliminary data indicate that co-culture with DD cells increased *CCL2, TNF, IL1B, IL6, IL10* and *FN* expression levels in THP-1 cells, whereas co-cultures with CT (control) cells had modest or no discernible effects on the expression of these genes. *TGFB* expression levels were unaffected by co-culture with either cell type.

CONCLUSIONS: If confirmed in ongoing analyses of myofibroblasts derived from additional patients, these findings would suggest that DD myofibroblasts secrete as-yet-unidentified factor(s) that "activate" THP-1 monocytes. Identification of these factors, which may be unique to fibrosis-associated myofibroblasts, could provide novel targets for therapeutic interventions designed to attenuate the progression and/or recurrence of DD and potentially other fibroses.

Poster session on Friday (F)

Connective tissues in development and disease

F1.

Differential regulation of osteoclast fusion and growth

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BACKGROUND: Osteoclasts are bone cells uniquely capable of a complete destruction of mineralized tissues. Osteoclasts are required for proper bone shaping and tooth eruption; they are also responsible for bone destruction in degenerative, inflammatory and metabolic bone disorders. Osteoclasts are formed by fusion of monocytes, cells of hematopoietic origin. A single osteoclast can contain between 3 and 100 nuclei, varying in diameter between 10 and 500 μM. It has been demonstrated that large osteoclasts are more likely to be observed during pathological bone resorption, and are more active and more responsive to environmental stimuli.

AIM: The aim of this study was to perform a secondary analysis of data collected in our laboratory to determine the pathways regulating osteoclast fusion and/or fusion-independent osteoclast growth.

Methods: The data from the studies that examined osteoclast differentiation from RAW 264.7 monocytic cells, or primary bone marrow or spleen derived osteoclast precursors were included. In these studies, osteoclasts were identified as TRAPpositive cells containing more than 3 nuclei, and were further characterized by image analysis using PixeLINK Capture SE® software and Image J. The cell surface area and nuclei number of 20–100 osteoclasts/condition were evaluated.

RESULTS: Large multinucleated osteoclast are first formed through fusion of monocytic cells, then later expanded in their overall size. Large number of treatments, including energy substrates, such as pyruvate, oxidative compound, such as ascorbic acid, and cancer-derived factors such as peroxiredoxin 4 and L-plastin, simultaneously increased osteoclast number, size and nucleation. Similarly, a number of inhibitors of different signaling pathways, including calcium, PKC, p38 and mTOR, simultaneously reduced osteoclast number, size and nucleation. However, in several instances we observed differential effects of inhibitors on osteoclast number/nucleation and size. Inhibition of ERK decreased osteoclast size, but increased surface area/nucleus, indicating that ERK is involved in regulation of osteoclast fusion, but not growth. Inhibition of AKT strongly reduced osteoclast nucleation, but not size, and increased surface area/nucleus, indicating that AKT is involved in osteoclast fusion but not growth. Inhibition of oxidative phosphorylation with low concentrations of sodium azide dramatically reduced osteoclast size, but not the number of nuclei/osteoclast, indicating that the cell growth is much more energy-expensive than fusion. In keeping, inhibition of AMPKγ strongly increased osteoclast size.

CONCLUSION: We identified ERK and AKT as mediators of monocyte fusion that do not affect osteoclast growth; and parameters of energy metabolism as specific mediators of osteoclast growth. Osteoclasts of larger size are implicated in pathological bone resorption. A better understanding of the regulation of osteoclast size is important for development of selective inhibitors that targets osteoclasts that are actively engaged in pathological bone destruction, while preserving physiological levels of osteoclast activity.

F2.

The expression of CCL18 as a unique marker of M2 macrophage in fibrotic lung diseases

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RATIONALE. Pulmonary fibrosis is an irreversible fibrotic lung disease that is characterized by myofibroblasts accumulation and tissue scarring that leads to a progressive increase in lung stiffness and a decline in its function. M2 macrophages, also known as alternatively activated macrophages, are believed to play a role in fibroproliferative disease as they possess profibrotic characteristics. It has been shown that CCL18 is excreted at higher levels in IPF patients. In-vitro, we have previously exhibited the ability to polarize monocytes from a human cell line, THP1, with the interleukin-4/interleukin-13/interleukin-6 (IL4/IL13/1L6) cocktail. This differentiation was confirmed through measurement of supernatant levels of Chemokine Ligand 18 (CCL-18), a secreted marker of the M2 phenotype. The aim of our study is to investigate the expression of CCL18 at the RNA level in M2 macrophages as well as in other cell population in different Interstitial lung diseases using the Nanostring gene-analysis technology and Single-cell RNA Sequencing (scRNAseq).

METHODS. A Tissue Microarray (TMA) was designed and generated from human lung tissues that were obtained from a tissue biobank for Interstitial lung diseases. It includes idiopathic pulmonary fibrosis (IPF), Hypersensitivity pneumonitis (HP), Sarcoidosis, Rheumatoid arthritis-ILD (RA_ILD) and Systemic sclerosis-ILD (SSc-ILD) and control lung tissues as well as PBMCs. Slides were cut in serial sections and stained with Immunohistochemical stains including CD68 and CD206. Duplex automatic RNAScope® *in situ* hybridization was performed on the human TMA to co-localize CD68 (macrophages marker), CCL18 (M2 marker) and PITPNM3+ (CCL18 receptor) at the RNA level. Tissue samples were taken from the parents' blocks (FFPE blocks) for RNA extraction and gene analysis using the Nanostring technology. scRNAseq was performed on control and IPF tissues.

RESULTS. Immunohistochemical assessment of CD206 positive cells indicated a statistical increase of CD206 in patients diagnosed with IPF, compared to control subjects ($p<0.05$) Using serial sections of the same region we show that CD68+/CD206+ positive regions are also positive for CCL18 CD68 and PITPNM3+ mRNA levels in human IPF tissue. Nanostring gene expression analysis shows high levels of CCL18 expression across all fibrotic diseases assessed. scRNAseq data show a high level of CCL18 expression in macrophages and at a lower level in other cells population.

CONCLUSION. CCL18 is highly and non-specifically expressed in alternatively activated macrophages in a variety of interstitial lung fibrotic disease. The receptor PITPNM3 is expressed both in macrophages and in epithelial cells and may offer insight into the biological role of CCL18. This data suggests a common role of alternatively macrophages and CCL18 in pulmonary fibrosis.

F3.

Osteocalcin knock-out restores glucose metabolism to wild type levels in a mouse model of osteogenesis imperfecta

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OBJECTIVE: Osteogenesis imperfecta (OI), mainly caused by genetic mutations in the bone matrix protein collagen type I, is characterized by low bone mass and high fracture rate. Recently, we have shown that in addition to bone fragility, growing mice with a severe form of OI, *Col1a1Jrt/+* mice, exhibit altered glucose/insulin metabolism and energy expenditure. We demonstrated significantly elevated serum levels of undercarboxylated osteocalcin (uOCN), a novel bone-derived hormone that affects insulin production and sensitivity, in these mice. To further confirm the role of uOCN, we crossed *Col1a1Jrt/+* mice (OI), on FVB background, with mice lacking one or both osteocalcin genes (OCN+/-, OCN-/-), on C57/BL6 background, to generate OI/OCN mice on mixed background.

METHODS: At an age of 4 and 8 weeks, wild-type (WT/WT) and OI/OCN mice were phenotypically characterized and glucose tolerance test was performed.

RESULTS: OCN-/- mice are infertile, wherefore OCN+/- mice were used for crossing experiments. Within the first generation, about 31% of generated pups were WT/OCN+/-, 24% OI/WT, 22% either OI/OCN+/- or WT/WT. One percent of mice were found dead. OI/OCN+/- mice of the first generation were used for further breeding. The second generation revealed a genetic distribution of about 32% WT/WT, 16% OI/OCN+/- or WT/OCN+/-, 12% OI/OCN-/-, and 4% WT/OCN-/- or OI/WT. Sixteen percent of mice in the second generation were found dead. Compared to WT/WT, mice harboring the genotype OI/WT, OI/OCN+/-, and OI/OCN-/- were smaller in size and up to 25% lower in body mass, which persisted during growth. At 4 weeks of age, OI/OCN+/- mice exhibited improved glucose tolerance while OI/OCN-/- mice did not differ from WT/WT littermates. However, at 8-weeks of age, no significant differences in glucose tolerance was found in OI/OCN+/- or OI/OCN-/- mice compared to WT/WT.

CONCLUSION: In *Col1a1Jrt/+* mice, genetic inactivation of one or both osteocalcin genes had no effect on body mass. However, knock-out of both osteocalcin genes restored glucose tolerance to WT levels in *Col1a1Jrt/+* mice, strongly supporting the causative role of osteocalcin in driving alteration in glucose/insulin metabolism in OI mice.

F4.

Exploring the pathophysiological cell signalling pathways induced by the osteoblast protein BRIL

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Osteogenesis imperfecta (OI) is a heritable disorder that affects bones and leads to overall skeletal fragility and frequent fractures in children. The genetic cause of OI type V is a recurrent heterozygous c.-14C>T point mutation in the 5'-UTR of the BRIL gene. It causes a novel in-frame translation start site (ATG) that adds 5 amino acids (MALEP) onto the N-terminus of the BRIL osteoblast-specific membrane protein. Another dominant *BRIL* mutation, introducing a single amino acid change (serine to leucine) at position 40 of the protein (S40L-BRIL), was found to cause atypical OI type VI. It is hypothesized that the two mutant forms of BRIL have different mechanisms which lead to distinct forms of OI. The specific mechanisms by which the mutant proteins cause these disorders has yet to be ascertained, although a gain-of-function has been proposed. We hypothesize that the mutant BRIL proteins act by activating intracellular signalling pathway(s) leading to perturbed osteoblast function.

To explore this possibility, we screened by transient transfection in MC3T3-E1 osteoblasts whether BRIL could activate luciferase (Luc) reporters driven by multimerized copies of binding sites for 22 individual transcription factors. We found that BRIL robustly activated MEF2-Luc and NFATC-Luc in MC3T3-E1 and MLO-A5. Induction of MEF2-Luc by BRIL was also detected in human SaOS-2 and rat UMR106 osteosarcoma cells, but not in HEK293 and NIH3T3 cells, thereby demonstrating that BRIL's activity is limited to bone cells. The activity elicited by BRIL were comparable to those induced by expression of MEF2C and NFATC1 and acted synergistically when combined. MEF2-Luc activation by BRIL was dose-dependent and could be suppressed by co-expression of the MEF2 antagonist HDAC4. As compared to WT-BRIL, MALEP-BRIL showed consistently high induction levels in MLO-A5 cells, while S40L-BRIL significantly lower induction levels. Mechanistically, BRIL did not affect MEF2 or NFATC gene expression, protein expression and nucleo-cytoplasmic localization. Various modulation experiments using proteins involved in the PKA and calcium-related cell pathways revealed potential axes by which BRIL's effect is transmitted downstream to transcription factors. Structure-function studies showed that disruption of BRIL first five amino acids, whether by the presence of tags, amino acid deletion or alanine scanning resulted in inhibition of MEF2C and NFAT's inductions, thereby pointing to BRIL's function at its N-terminal. High amino acid conservation also hints at the same region of the protein having an important function. Specifically, disruption of D2, T3 and Y5 showed the greatest effect on luciferase readouts in MC3T3 cells.

In sum, clarifying BRIL's function will be of value to elucidate the functions specific to MALEP-BRIL and S40L-BRIL. Furthermore, methods to target the neomorphic function of MALEP-BRIL and S40L-BRIL mutants may lead to new avenues for therapeutic applications for OI.

F5.

Characterization of Wilms' tumour 1 (WT1) as a fibrotic biomarker for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most commonly inherited pediatric muscle disorder. DMD has no cure, and most patients succumb to the disease in their mid-twenties. It is characterized by muscle degeneration, resulting from the loss of the cytoskeletal protein dystrophin. Poor dystrophin function, or reduced production, leads to a decrease in structural support in muscle cells. These cells become prone to apoptosis, resulting in remodeling of muscle into fibrous and fatty connective tissue, impeding overall muscle function. Fibrosis is profound in DMD, generating a microenvironment that is a significant impediment to both endogenous muscle repair and any potential regenerative strategy. At present, there are few therapies that specifically target fibrosis and microenvironment improvement, but one possibility rests in targeting the Wilms Tumor 1 (WT-1) protein. WT-1 is a zinc finger transcription factor commonly found in nephroblastomas, and recently shown to be expressed in fibrotic conditions such as Dupuytren's disease and pulmonary fibrosis.

The objective of this research project is to characterize WT-1 expression in DMD muscle tissue. We hypothesize that WT-1 is upregulated in DMD muscle tissue prior to onset of fibrosis.

To provide an understanding of WT-1 function in DMD, our methodology makes use of wildtype control mice and mdx DMD model mice. Subtypes of these mdx mice, with one or both alleles for utrophin knocked out, and at young, mature, and aged timepoints, were also used to provide models for different fibrosis forms. We utilized immunohistochemistry to quantify WT-1 protein expression, and Masson's Trichrome staining to quantify fibrosis, in muscle tissue taken from the heart, diaphragm and gastrocnemius of these mice. One-way ANOVA with Bonferroni's post-hoc test was used to identify significant differences between groups.

WT-1 expression was found to be elevated in the diaphragm of mdx mice compared to wildtype controls, but not in the gastrocnemius muscles of these same mice. Furthermore, WT-1 expression was found to be elevated in both diaphragm and gastrocnemius muscles of mdx/utrn+/- and mdx/utrn-/- mice compared to wildtype controls. This effect was found in young and mature mice, but no effect was seen in aged mice. Only the mdx/utrn+/- and mdx/utrn-/- gastrocnemius and diaphragm were found to be fibrotic in mature mice, and these in addition to the mdx diaphragm were found to be fibrotic in aged mice.

Therefore, the hypothesis that WT-1 is upregulated in DMD muscle tissue prior to fibrosis was supported. These results suggest that WT-1 is a broad marker for fibrotic conditions, rather than being limited to conditions such as pulmonary fibrosis. With regards to future work, the impact of WT-1 on the progression of fibrosis still requires investigation. Clinical applications involving WT-1 are numerous, most notably the foremost application that WT-1 may be used as a biomarker to detect the onset of fibrosis. Additionally, immunotherapies currently exist which target WT-1 positive cancer cells for destruction by the immune system. Should WT-1 contribute to fibrosis rather than only acting as a biomarker for it, whether the application of these therapies impedes fibrosis overall can be examined.

F6.

Novel c.G630A TCIRG1 mutation causes aberrant splicing resulting in an unusually mild form of autosomal recessive osteopetrosis

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Autosomal recessive osteopetrosis (ARO) is a severe genetic bone disease characterized by high bone density due to mutations that affect formation or function of osteoclasts. Mutations in the *a*3 subunit of the vacuolar type H+-ATPase (encoded by *TCIRG1*) are responsible for ~50% of all ARO cases. We identified a novel *TCIRG1* (c.G630A) mutation responsible for an unusually mild form of the disease. To characterize this mutation, osteoclasts were differentiated using peripheral blood monocytes from the patient (c.G630A/c.G630A), male sibling (+/+), unaffected female sibling (+/c.G630A), and unaffected parent (+/c.G630A). Osteoclast formation, bone resorbing function, TCIRG1 protein and mRNA expression levels were assessed. The c.G630A mutation did not affect osteoclast differentiation; however, bone resorbing function was decreased. Both TCIRG1 protein and full-length *TCIRG1* mRNA expression levels were also diminished in the affected patient's sample. The c.G630A mutation replaces the last nucleotide of exon 6 and may cause splicing defects. We analyzed the *TCIRG1* splicing pattern between exons 4 to 8 and detected deletions of exons 5, 6, 7, and 5-6 (DE56). These deletions were only observed in c.G630A/c.G630A and +/c.G630A samples, but not in +/+ controls. Among these deletions, only DE56 maintained the reading frame and was predicted to generate an 85 kDa protein. Exons 5-6 encode an uncharacterized portion of the cytoplasmic N-terminal domain of *a*3, a domain not involved in proton translocation. To investigate the effect of DE56 on V-ATPase function, we transformed yeast with plasmids carrying full-length or truncated Vph1p, the yeast ortholog of *a*3. Both proteins were expressed; however, DE56-Vph1p transformed yeast failed to grow on Zn2+-containing plates, a growth assay dependent on V-ATPase-mediated vacuolar acidification. In conclusion, our results show that the DE56 truncated protein is not functional, suggesting that the mild ARO phenotype observed in the patient is likely due to the residual full-length protein expression.

F7.

Murine mouse model for Osteofibrous Dysplasia

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Osteofibrous dysplasia (OFD) is a rare disease accounting for 0.2% of all primary bone tumours. It is characterized by the development of fibrous dysplasia, having a predilection for the tibia, where it causes non-healing tibial fractures. There is currently no cure for OFD and treatment is limited to the insertion of surgical rods to help support the non-healing fractures. Our lab has shown that OFD is secondary to gain-of-function mutations in the receptor tyrosine kinase mesenchymal epithelial transition (MET) gene. Fracture tissue samples from affected patients exhibit aberrant MET activity and delays in osteoblast differentiation. Here we look to utilize a genetic mouse model which replicates the MET mutation seen in human OFD patients in order to further study the disease. Utilizing whole mount skeletal preparations, we have been able to demonstrate no gross defects in our mouse model. Furthermore, through histological staining, we have demonstrated no gross defects in epiphyseal growth plates in our genetic mouse model. Therefore, this leads us to believe the osteoblast differentiation defect discovered is a stress induced defect. We will be examining this hypothesis through simple closed transverse fracturing model and examining the progression of fracture healing in mice. Children affected by OFD experience severe pain and disabilities due to non-healing fractures, resulting in gait deformation and punishing reduction in quality of life. The role of MET in bone formation is unstudied, therefore we will be one of the first groups to elucidate MET's role in fracture healing. The use of a critical tibial defect model is transformative as many groups have failed to simulate human conditions using simplistic models. Our mouse model allows direct translation to patient care and will improve affected patients' quality of life.

F8.

Dendritic Cell-Specific Transmembrane protein (DC-STAMP) deficiency ameliorates inflammation and joint damage in TNF-driven experimental arthritis

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BACKGROUND: Pathologic bone resorption in rheumatoid arthritis (RA) is mediated by multinucleated osteoclasts (OC) at the bone-pannus junction. OC differentiation (OCgenesis) is dependent on a number of signals that promote cell fusion, formation of specific organelles and the development of a ruffled border. Dendritic Cell-Specific Transmembrane Protein (DC-STAMP), is upregulated in monocytes at early stages of OCgenesis and is required for cell-cell fusion. DC-STAMP-/- mice develop mild osteopetrosis, due to the presence of mononuclear OC with limited capacity to resorb bone. The role of DCSTAMP in the induction of joint inflammation and pathologic bone resorption has not been examined. Therefore, we determine whether the absence of DC-STAMP ameliorates joint inflammation and bone damage in the TNF transgenic murine arthritis model.

METHODS: Experimental groups comprised: 5-month old C57BL/6, DC-STAMP-/-, TNF-Tg and newly generated DC-STAMP-/- x TNF-Tg female mice. The presence of arthritis was determined by visual examination and measuring ankle width with engineer's caliper. We determined the area occupied by OC in bone sections by TRAP staining, and evaluated the impact of DCSTAMP deficiency on bone resorption with mCT-scan analysis. Serum cytokines were measured by multiplex assay and the accumulation of inflammatory cells in bone sections was quantitated by flow cytometry and immunofluorescence.

RESULTS: DC-STAMP-/- x TNF-Tg mice had a significant 2.7-fold decrease in ankle thickness from week 17 to 20 of age, compared to age-matched TNF-Tg mice ($p<0.001$). Notably, DC-STAMP-/- x TNF-Tg mice had a significantly smaller TRAP+ area in the tibia and femur, compared to the TRAP+ area in the tibia (1.05 ± 0.1 vs 25.3 ± 0.4, $p=0.001$) and femur (7 ± 0.2 vs 38 ± 0.5, $p=0.05$) of TNF-Tg mice. Consistent with the remarkable reduction in OC in the femur and tibia, we found a significant 1.6-fold reduction (femur) and four-fold reduction (tibia) in bone resorption by OCs in DCSTAMP-/- x TNF-Tg mice, compared to TNF-Tg mice (10 ± 0.03 vs. 6.0 ± 0.01, $p=0.001$ and 5.3 ± 0.5 vs. 21 ± 0.05, $p=0.002$). Accumulation of inflammatory cells in the synovia, and systemic levels of TNF-α(20 pg/ml, 30-fold decrease, $p=0.04$) and MCP1 (56 pg/ml, 3-fold decrease, $p=0.04$) were markedly reduced in DC-STAMP-/- x TNF-Tg mice. In agreement with these findings, macrophage infiltration was abundant in the synovial tissue from TNF-Tg mice and correlated with the significantly higher levels of MCP1 (170 pg/ml), a chemokine that attracts macrophages into inflamed tissues.

CONCLUSIONS: We found that DC-STAMP is required for progressive development of synovitis and joint damage in the setting of TNF-driven arthritis. Apparently, DC-STAMP modulates synovitis and joint damage by regulating recruitment of OC precursors and possibly other immune cells.

F9.

The prevalence and distribution of missing and unerupted teeth in 171 patients with osteogenesis imperfecta

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Osteogenesis imperfecta (OI) known as "Brittle Bone Disease," is a genetic disorder characterized by decreased bone mineral density (BMD), skeletal dysplasia, increased bone fragility. Additional secondary symptoms associated with OI include blue sclerae, hearing loss, dentinogenesis imperfecta (DI), pulmonary deficiency, and cardiac valvular dysfunction. A significantly high incidence of impacted second molars and twice as many missing teeth as the general population was reported in OI. Missing and unerupted teeth in osteogenesis imperfecta have been minimally studied. With a small number of individuals, and only on certain teeth. The objective of this study is to describe the prevalence and incidence of missing and unerupted teeth in a big cohort of OI. We hypothesized based on previous literature that every type of OI has specific and different patterned of both missing and unerupted teeth. Method: a cohort of 171 individuals with different OI types, were evaluated for missing and unerupted teeth using panoramic radiographs. Results: The mean of unerupted teeth in all OI patients is .8 ± 1.7, while for missing teeth is 2.41 ± 15. Patients with OI type III showed the highest prevalence of Missing and Unerupted teeth (62%,70%) followed by OI type IV (52%-40%) with no significant difference, and finally OI type I (11%-3%). Individuals with OI type I have significantly lowered then OI type III and IV in the prevalence of missing and unerupted teeth. Premolars were the most missing teeth in all OI types and in OI type III and IV they were missing in 30% of the patients. However, tooth 17 were unerupted in about 60% of patients with OI type III and IV. Followed by the lower second premolars. Conclusion: Missing teeth are associated with a more severe form of OI. A unique feature associated with OI is the high prevalence of missing premolars in all OI types. Unerupted teeth were mostly found in individuals with OI type III and IV, with the high prevalence in upper second molars and lower second premolar.

Poster session on Friday (F)

Mechanisms of Matrix Remodeling and Disease

F10.

Expression levels of F13A1 transglutaminase in adipose tissue significantly associates with metabolic health in obesity

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The prevalence of obesity − where excess body fat accumulates – increases the risk of developing type 2 diabetes, and other comorbidities including arthritis, cardiovascular diseases, and several types of cancers. Although, majority of obese individuals develop comorbidities, it is estimated that 10-25% of obese humans remain healthy. This *metabolically healthy obesity* (MHO) has been speculated to arise from protection from development of insulin resistance and altered adipose tissue properties of individuals. Adipose tissue is a form of connective tissue that is capable of storing lipids. Its normal function is essential to stablilize energy metabolism and general health and it is considered that metabolic disturbances in obesity arise largely from adipose tissue failure. To investigate adipose tissue-genes causative to obesity and metabolic health, Pietiläinen group examined genome wide gene expression in adipose tissue and adipocytes of monozygotic Finnish, body mass index (BMI)-discordant twins, i.e. lean-obese twin pairs. The analyses identified 27 potentially causative genes in the twin pairs; out of these, the gene with strongest causative relation to obesity was *F13A1* which encodes for Factor XIIIa transglutaminase. Our recent work FXIII-A enzyme has demonstrated that its deletion in mice results in characteristics of MHO and our further studies suggest it regulates insulin sensitivity *in vivo* and adipocyte proliferation *in vitro*. Interestingly, Pietiläinen group also reported that among the monozygotic twin pairs had clear groups that demonstrated metabolic differences; MHO-Group 1: insulin sensitive, low liver fat% in obese twin, and metabolically sick obesity MSO-Group 2: less insulin sensitive, high liver fat% in obese twin. Here we have used the twin GWA/Affymetric mRNA and metabolic data to examine *F13A1* mRNA levels and correlation with possible markers of adipogenesis, tissue expansion and metabolic disturbances. We performed statistical analyses of the data set (52 individuals, 26 twins) by means of testing, correlations, and descriptive statistics, in the the MHO-Group 1 and MHS-Group 2 data as well as in BMI-based scrambled data set. We report that *F13A1* mRNA levels are significantly higher in obese twins and highest in the obese twins of the MHS group. Conversely, lowest averaged levels were observed in the lean twins of MHO group. Adipose tissue cellularity in the MHO group was significantly higher compared to MSO where adipose tissue weight was significantly higher, suggesting different mode of expansion that may be genetically regulated. *F13A1* expression levels were also significantly higher in obese scrambled data (BMI above 30) versus normal BMI. The data suggests that *F13A1* levels in adipose tissue link to metabolic health outcome of obesity and may be linked to adipose tissue capacity to expand in and to respond to energy surplus.

F11.

Investigating CCN family proteins as potential therapeutic target in Duchenne muscular dystrophy

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BACKGROUND: Duchenne Muscular Dystrophy (DMD) is a severely debilitating neuromuscular disorder caused by the absence of dystrophin leading to progressive muscle degeneration. Currently, the disease affects approximately 30, 000 male children, most of whom succumb to the disease by their mid-twenties. This fatal disorder is characterized by fibrotic microenvironments, which are a severe impediment to both endogenous muscle repair and any potential regenerative strategy. The CCN protein family is unified by their unique structure and structure dependent functionality. CCN1/CYR61, CCN2/CTGF and CCN3/Nov are matricellular proteins known to be associated with extracellular matrix manipulation, cell growth regulation, and cell differentiation. Reducing CCN2 levels in a DMD murine model is known to improve muscle repair therapy and reduce fibrosis progression. CCN2 mediates differentiation of progenitor cells into myofibroblasts and the transforming growth factor (TGF)-β pathway to induce collagen synthesis. Once initiated, fibrosis is maintained by proliferation of myofibroblasts and matrix stiffness. Preliminary data suggests that CCN1 is required for maintaining matrix stiffness via enzyme expression that promote collagen crosslinking and stability. Generally, CCN3 has a complex context dependent expression and function based on interactions with surrounding proteins and tissue-type. Previous studies demonstrated CCN3 induces muscle skeletal cell proliferation and survival through integrin-based interactions. In addition, studies have shown CCN3 has antagonistic effects to CCN1 and CCN2 in TGF- β mediated pathways by reducing collagen production and deposition in skin and kidney. However, a better understanding of the involvement of CCN family members in DMD is necessary to determine their potential as a therapeutic target.

HYPOTHESIS AND OBJECTIVE: We hypothesize that CCN1 and CCN3 levels correlate to the severity of fibrotic microenvironments and muscle degeneration in DMD murine models. We hope characterization of the role of CCN1 and CCN3 will help us gain a better understanding of the underlying mechanisms behind fibrosis and muscle repair in DMD.

METHODS: We investigated, using immunohistochemistry and western blot, CCN3 levels in skeletal muscle of mdx, mdx/utr +/-, mdx/utr -/-, and wild-type model over three time points – 4 to 5 weeks, 8 to 10 weeks and 40+ weeks. The mdx/utr -/- model is the closest representation of fibrosis severity and progression found in DMD human patients.

RESULTS AND DISCUSSION: Using *ex vivo* histological analysis, we found CCN3 expression appeared in a heterogenous pattern across the mouse skeletal muscle tissue mainly found along individual muscle fibers. In addition, CCN1 expression predominantly in the extracellular matrix of mouse skeletal muscle tissue. Our preliminary data showed CCN3 levels increased in the mdx/utr -/- and mdx model. In contrast, CCN3 levels decreased in the skeletal muscle of the mdx/utr +/- model. Our preliminary data showed no difference in CCN3 levels between the various time points within each model.

CONCLUSION: Considering CCN1 and CCN3 expression share similar features to CCN2 expression in the skeletal muscle of DMD murine models, our research demonstrates potential for new therapeutic strategies for DMD.

F12.

Examining the role of Dectin-1/Clec-7a as a marker of alternatively activated macrophages in idiopathic lung fibrosis

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RATIONALE: Idiopathic Pulmonary Fibrosis (IPF) is one of the major forms of interstitial lung disease (ILD) characterized by a progressive decline in lung function with undetermined pathogenesis. Although a role of alternatively activated macrophages (AAM) in fibrotic pathogenesis has been suggested, their specific contribution is not fully understood. Previously, we have identified Dectin-1/Clec7a as a unique AAM marker in both murine and human systems. The current study further characterizes the expression of Dectin-1/Clec7a in IPF and various fibrotic lung diseases and investigates the exact role of this pathway in AAM macrophages in a murine model of lung fibrosis. We hypothesized that the Dectin-1/Clec7a pathway contributes to accumulation of fibrotic AAM macrophages and fibrogenesis.

METHODS: To assess Dectin-1/Clec7a in IPF patients, a human tissue microarray (TMA) was generated from archived formalin-fixed paraffin-embedded (FFPE) tissues from 24 IPF patients and 17 control subjects. TMA cores were selected from both fibrotic and non-fibrotic regions. To validate the expression of Dectin-1/Clec7a in various fibrotic lung diseases, a subsequent TMA was developed containing cores derived from patients diagnosed with IPF, HP, RA, Sarcoidosis and control (6 subjects per group). Masson's trichrome staining, immunohistochemistry and fluorescent in situ hybridization was completed to assess Dectin-1/Clec-7A expression. To examine the role of this pathway, Dectin-1/Clec7a knockout mice were exposed to low dose bleomycin for 16 days in an experimental mouse model of lung fibrosis. Lung elastance was measured using flexivent analysis and macrophage populations were assessed using flow cytometry.

RESULTS: Dectin-1/Clec7a expression was significantly increased in fibrotic regions ($p<0.05$) compared to non-fibrotic regions of IPF patients and control subjects and we validated the expression of Dectin-1/Clec7a in various lung diseases. Surprisingly, Dectin-1/Clec7a knockout mice demonstrated an increase in lung elastance in response to bleomycin compared to controls and wildtype mice ($p<0.05$). There was no significant difference in elastance in wildtype mice compared to controls. Clec7a-/- mice exhibited a significant accumulation of both MHCII+CD64+ activated macrophages and AAM macrophages characterized by expression of Arginase-1 and CD206 compared to wildtype ($p<0.05$). Interestingly, wildtype mice expressed higher levels of Clec7a+ AAM macrophages in response to bleomycin compared to controls ($p<0.01$).

CONCLUSION: In conclusion, Dectin-1/Clec7a is a novel marker for AAM macrophages involved in IPF. Further, AAM macrophages may have bidirectional roles based on presence of Dectin-1/Clec7a and our data suggests a protective role of Clec7a+ AAM macrophages in lung fibrogenesis.

F13.

Role of matrix metalloproteinase-9 in extracellular matrix remodeling in disease

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The extracellular matrix (ECM) is the highly dynamic noncellular structure that provides the physical scaffolding crucial for maintaining tissue architecture and the biochemical signaling necessary for homeostasis. The ECM undergoes constant deposition and degradation in the process of ECM remodeling in response to stressors, tissue needs, and biochemical signals that are mediated primarily by matrix metalloproteinases (MMPs) which work to degrade and build up the ECM. Abnormally high ECM deposition has been associated with fibrosis and cancer, while excessive ECM degradation has been linked to the development of osteoarthritis. Research on MMP-9 has highlighted its crucial role in the process of ECM remodeling, maintaining tissue architecture and regulating homeostatic functions. This is perhaps not surprising due to MMP-9 expression on the surface of many cell types, existing in complex with G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) or Toll-like receptors (TLRs). Through a novel, yet ubiquitous signaling platform, we have shown that MMP-9 is not only involved in the direct remodeling of the ECM but also in the transactivation of associated receptors to mediate and recruit additional remodeling proteins. As a tripartite complex on the cell surface, we hypothesize the implications of MMP-9 associated with the TrkA receptor, TLRs, epidermal growth factor receptor (EGFR) and the insulin receptor (IR) as they contribute to various aspects of ECM remodeling. These newly discovered roles of MMP-9 provide additional therapeutic targets for the treatment of several pathological conditions including cancer, atherosclerosis, diabetes, inflammation, and wound healing.

F14.

Roles of RELMα/FIZZ1 in OSM-mediated lung inflammation and ECM accumulation

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INTRODUCTION: Resistin-like molecule alpha (RELMα)/found in inflammatory zone 1 (FIZZ1) is a cysteine-rich secreted protein associated with Th2-skewed inflammation and implicated in mouse models of bleomycin-induced pulmonary fibrosis, experimental asthma, and parasitic infection. We have previously shown that transient over-expression of Oncostatin M (OSM) in mouse lung induces Th2-skewed cytokine profiles, extracellular matrix (ECM) deposition, and accumulation of alternatively activated (M2) macrophage populations, which are key producers of RELMα in certain mouse models. Here, we evaluate the regulation of RELMα by OSM *in vivo*, and examine the role of RELMα in OSM-induced lung inflammation and ECM accumulation using RELMα-deficient mice.

METHODS: Wild-type and RELMα–/– C57Bl/6 mice were endotracheally administered PBS, control adenoviral vectors (AdDel70), or those expressing mouse OSM (AdOSM) to induce transient over-expression in the lung. After 7 days, mRNA and protein were assessed by Nanostring, Western blot or ELISA for various analytes. Formalin-fixed paraffin-embedded lung tissues were examined by chromogenic *in situ* hybridization (CISH) and H&E staining. Broncho-alveolar lavage (BAL) was assessed for inflammatory cell accumulation.

RESULTS: RELMα gene and protein expression was detectable at basal levels in whole lung, BAL and serum of naïve and AdDel70-treated wildtype mice (100ng/mL in BAL and 200ng/mL in serum), and was significantly elevated upon AdOSM treatment at day 7 (ranging from 10000–12000ng/mL in BAL and 400–800ng/mL in serum). Transient over-expression of OSM in wild-type mice resulted in thickening of the airway epithelium, as assessed by H&E staining of lung tissue, and upregulation of ECM remodeling genes *Col1a1*, *Col3a1*, *Mmp13*, and *Timp1* ($p < 0.001$) which was significantly suppressed in AdOSM-treated RELMα-deficient mice (20-50% reduction, $p < 0.05$). However, RELMα deficiencies did not affect the AdOSMmediated induction of OSM, nor of Th2-skewed mediators such as IL-4, IL-5, IL-6, nor of eosinophil accumulation in the BAL. CISH staining of RELMα and CD68 showed that RELMα was not expressed in CD68+ macrophages, but predominantly expressed in columnar airway epithelial cells. Furthermore, the absence of RELMα led to reduced numbers of CD206+ M2 macrophages in total lung (50% reduction) in comparison to wild-type mice treated with AdOSM, as assessed by flow cytometry.

CONCLUSION: Together, the results show that OSM induces RELMα markedly in columnar epithelial cells, and that M2 macrophage accumulation induced by OSM *in vivo* and OSM-induced ECM remodeling may act in part through RELMα.

F15.

The role of SMOC2 in renal cell carcinoma

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INTRODUCTION: Kidney cancer is among the 10 most frequently occurring cancers in Canada. Approximately 90% of kidney cancers are characterized as Renal Cell Carcinoma (RCC), which is typically asymptomatic at early stages, prone to metastasis and has a poor prognosis at advanced stages. The epithelial-mesenchymal transition (EMT) is a genetic program that promotes metastatic dissemination of malignant cells from primary epithelial tumors. During EMT, cells lose their epithelial phenotype by reorganizing their adhesion and cytoskeletal structures to acquire a mesenchymal morphology and the ability to migrate. The extracellular matrix (ECM) surrounding a cancer cell has been proven to be essential in tumor progression by directly promoting cellular transformation and metastasis. Matricelluar Proteins (MPs) are ECM-regulating proteins that modulate cell-matrix interactions to influence cellular behavior and function. MPs also exhibit contextual expression, typically induced transiently during repair that can be sustained by malignant and/or tumor-associated cells. We have previously characterized SPARC-Related Modular Calcium-Binding Protein 2 (SMOC2) to be highly expressed during processes involving tissue remodeling with emerging roles in cancers, yet its role in RCC remains elusive.

HYPOTHESIS: We believe that SMOC2 is a key signalling molecule from the secretome of an RCC tumor that can mediate ECM-induced tumor progression and metastasis. Our goal is to investigate SMOC2 expression in RCC and if it can promote EMT metastasis in RCC cell lines.

METHODS: 1) Whole genome RNA sequencing and immunohistochemistry staining of RCC patient biopsy samples for SMOC2 analysis. 2) In vitro cell culture treatment with recombinant SMOC2, and transfections with SMOC2 vector or siRNA in RCC human cell lines 786-O. Normal kidney cell lines HK-2 and MDCK were used as controls. Cell lysates analysed by Western Blot for various EMT markers. 3) Functional assays performed on cell lines to observe the effect of SMOC2 during, polarity (Scratch assay) and migration (Boyden Chamber).

RESULTS: Using published gene expression profiles, we identified SMOC2 as being significantly expressed in RCC patients compared to normal renal tissue. Our analysis of *de novo* protein synthesis of SMOC2 is much higher in the tubular epithelial cells of patients with biopsy-proven RCC. Human RCC cell, 786-O, expressed higher endogenous SMOC2 levels than normal human kidney epithelial cells, HK2. Furthermore, recombinant SMOC2 treatment and overexpression in 786-O promoted expression of mesenchymal markers fibronectin, vimentin and α-SMA and downregulation of epithelial marker E-cadherin. On the contrary, opposite effects were observed with SMOC2 siRNA transfections. Further support of these EMT observations come from our functional assays which showed features of metastasis. Using a Scratch assay to test cell polarity, SMOC2-transfected MDCK cells led to loss of cell contact with extensions branching out and into the wound site typical of mesenchymal cells. Our transwell migration assay of SMOC2 overexpressing and recombinant-treated cells had increased migration.

CONCLUSION: Our present work shows that SMOC2 promotes metastatic behaviour of epithelial tumors thru EMT. Understanding how SMOC2 regulates ECM-cellular interactions and if their deregulation influences cancer progression and metastasis may help regard the tumor microenvironment as an accessible therapeutic and biomarker target.

F16.

Fibulin-4 and latent transforming growth factor-β binding protein-4 cell interactions in elastogenesis

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INTRODUCTION: Elastogenesis presents a cell surface located hierarchical process that requires the recruitment of several proteins, including fibulin-4 (FBLN4) and latent transforming growth factor beta binding protein-4 (LTBP4). Mutations in FBLN4 and LTBP4 cause autosomal recessive cutis laxa type B and C, respectively. Knockout mouse models of FBLN4 and LTBP4 emphasized the roles of these two proteins in elastogenesis. Previously, we showed that FBLN4 interacts with fibroblasts. Cell interaction with LTBP4 has also been demonstrated. However, the cell receptors for FBLN4 and LTBP4, and the respective molecular mechanisms in elastogenesis remain unknown. In this study, we aimed to identify the FBLN4 and LTBP4 cell receptors, and to determine the functional consequence of their cell interactions in elastogenesis.

RESULTS: Here, we show that skin fibroblasts and vascular smooth muscle cells bind strongly to FBLN4 and LTBP4 using realtime and end-point cell binding assays. Both, synthetic and contractile aortic smooth muscle cells interacted similarly with FBLN4 and LTBP4. We also demonstrate the functionality of FBLN4 multimerization in cell binding. FBLN4 multimers exclusively interact with cells, but not monomers. With a set of FBLN4 deletion mutants, we identified two cell interaction epitopes on FBLN4, one located in cbEGF2-3 and a second one in the C-terminal domain. FBLN4 glycosylation in cbEGF3 and the C-terminal domaindoes not affect cell binding.We also have investigated FBLN4 and LTBP4 cell receptor(s). Both FBLN4 and LTBP4 have high affinity for heparin. This suggests that heparan sulfate proteoglycans mediate FBLN4 and LTBP4 cell interaction. In the presence of heparin (an experimental model of heparan sulfate), cell binding to FBLN4 was entirely abolished, and reduced to LTBP4. Likewise, cellular interaction with FBLN4 and LTBP4 was reduced in the presence of heparan sulfate. Treating cells with heparinases significantly reduced cell attachment to both proteins. Heparan sulfate deficient cells did not bind to FBLN4. Information about the expressed heparan sulfate proteoglycan cell receptors in skin fibroblasts and vascular smooth muscle cells are required for the downstream identification of the FBLN4 and LTBP4 receptors. Therefore, real-time quantitative PCR assays were conducted to profile the mRNA expression levels for a number of cell surface receptor candidates in primary elastogenic cells including fibroblasts, smooth muscle cells. The expressions of syndecan-1 and glypican-1 were absent in some of the examined elastogenic cells while these cells interact strongly with FBLN4 and LTBP4. That excluded syndecan-1 and glypican-1 from the candidate list as cell receptors for FBLN4 and LTBP4. siRNA knockdowns were used to identify the FBLN4 and LTBP4 cell receptors. Bothsyndecan-2 and -3 knockdowns in fibroblasts abolished interaction with FBLN4, whereas only syndecan-3 knockdown abolished interaction with LTBP4. Syndecan-2 and -3 knockdowns in cultured skin fibroblast resulted in compromised elastic fiber assembly examined by immunofluorescence staining.

CONCLUSIONS: FBLN4 contains two cell interaction sites mapped to cbEGF2-3 and the C-terminal domain. FBLN4 interacts with syndecan-2 and -3, whereas LTBP4 interacts with syndecan-3.The data suggest a new cell-interaction role for FBLN4 and LTBP4 which is essential for proper elastogenesis.

F17.

CD109 differentially regulates ALK5 versus ALK1 signaling pathways and decreases collagen type II levels and proteoglycan content in articular cartilage in vivo in mice

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INTRODUCTION: Mature articular cartilage displays poor intrinsic healing and its degradation is the major hallmark of osteoarthritis (OA). TGF-β is a multifunctional cytokine that plays a critical role in cartilage repair and maintenance. Aberrant TGF- β signaling in chondrocytes has been strongly implicated in the pathogenesis of OA. Our group has previously reported CD109 as a novel TGF-β co-receptor and shown that CD109 is a potent negative regulator of TGF-β signaling in the skin.

OBJECTIVE: The proposed study is aimed at identifying whether manipulation of CD109 expression levels modulates the balance between TGF-β signaling via ALK1 versus ALK5 signaling pathways and regulates ECM protein expression *in vivo* in articular chondrocytes.

METHODS: Articular cartilage tissue was collected and primary chondrocytes were isolated from CD109 KO and wild-type mice. TGF-β signaling components were analyzed in isolated chondrocytes or cartilage tissue by determining ALK5 versus ALK1 levels and Smad2/3 versus Smad1/5 levels using Western blot. Chondrocyte function was determined by evaluating the expression of, collagen type II, aggrecan, collagenase (MMP-13) and aggrecanase (ADAMTS-5), at the protein and mRNA levels by Western blot, real time PCR or immunocytochemistry (ICC). Histological features and proteoglycan content of cartilage from CD109 KO and wild-type mice were assessed by Safranin O/fast green staining.

RESULTS: Articular chondrocytes isolated from CD109 KO mice display markedly enhanced levels of ALK5, and collagen type II, and increased expression of aggrecan, in comparison with chondrocytes from wild-type mice. On the other hand, loss of CD109 expression in mice articular chondrocytes results in decreased levels of ALK1, MMP13 and ADAMTS5. Moreover, histological results indicate that collagen content in articular cartilage from CD109 KO mice is increased significantly, as compared to cartilage from wild-type mice.

CONCLUSION: Our findings suggest that CD109 differentially regulates TGF-β signaling pathways and inhibits ECM protein production while promoting proteases expression, in articular chondrocytes *in vivo*. We conclude that CD109 may play an important role in maintaining cartilage function and integrity.

F18.

Microfibrillar-associated protein 4 (MFAP4) characterization and functional relevance in the elastic tissues

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INTRODUCTION: Microfibrillar-associated protein 4 (MFAP4) is a glycoprotein of the fibrinogen family, containing the fibrinogen C-terminal domain. MFAP4 is an extracellular matrix protein that is involved in cell adhesion and intercellular interactions. MFAP4 is also critical in formation of elastic fibres. MFAP4 binds to tropoelastin as well as fibrillin-1 and fibrillin- 2 in their N-terminal regions. Binding to other elastic fiber components, including lysyl oxidase, desmosine, insoluble elastin and type I collagen was also reported.

METHODS: Using surface plasmon resonance (SPR) spectroscopy (Biacore), we report here binding properties of MFAP4 to several elastogenic proteins including LTBP4, short fibulins and fibronectin. The MFAP4 structural characteristics were studied by atomic force microscopy (AFM) and negative staining transmission electron microscopy (TEM). MFAP4 distribution in human skin tissues was studied by immunofluorescence experiments.

RESULTS: Both AFM and negative staining TEM showed that MFAP4 predominantly occurs as particles highly homogenous in size with a flattened donut shape. The diameter is about 15 nm, and the height is 2-3 nm as determined by AFM. SDS-PAGE experiments showed that these particles represent dimers. This was confirmed by dynamic light scattering experiments that showed that 82% (mass percentage) of particles have sizes attributable to dimers (Rh=3.7 nm, molecular mass=72 kDa), 15.3% are octamers (Rh=6.6 nm, molecular mass=282 kDa), the remainder of 2.7% are high molecular mass oligomers (Rh=25.1 nm, molecular mass=6346 kDa). By SPR we first confirmed binding to the N terminal fibrillin-1 fragment rFBN1-N (KD=5.7 nM), and no binding to the C terminal fibrillin-1 half rFBN1-C. Surprisingly, we found that MFAP4 also binds via a second binding site to a central region (rF1M) of fibrillin-1 with high affinity (KD=6.4 nM). MFAP4 self-interacts with an affinity of KD=59 nM, binds relatively strongly to LTBP4 (KD=22 nM), and moderately to fibulin-4 (KD=121 nM) and fibulin-5 (KD=357 nM). MFAP4 does not bind to fibulin-3 and fibronectin. AFM experiments suggest that MFAP4 may form complexes with rF1M confirming the SPR data. Staining of human skin samples revealed strong co-localization of MFAP4 with elastic fibers.

CONCLUSIONS: In summary, we report here new aspects of MFAP4 properties with relevance to elastogenesis, the particle structural characteristics, and extend the MFAP4 interactome to key elastogenic proteins. These data are required to understand mechanistic aspects of MFAP4 in elastogenesis.

F19.

Characterizing the role of IRE1/XBP1 pathway activation in alternatively activated macrophages through molecular phenotyping in interstitial fibrotic lung disease

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BACKGROUND/ OBJECTIVES: Idiopathic Pulmonary Fibrosis (IPF), a disease of pathologic scarring, is the most prevalent idiopathic interstitial pneumonia. Although alternatively activated (M2) macrophages have been associated with fibrotic disease, their origin and exact mechanistic contribution are not fully understood. We have previously shown that the addition of the cytokine IL-6 to the profibrotic cocktail Il-4/IL-13 led to a drastic increase in Chemokine Ligand 18 (CCL-18) secretion in phorbol myristate acetate activated and polarized THP1 macrophages. This process was shown to be dependent on the activation of the Unfolded Protein Response (UPR) and endoplasmic reticulum (ER) expansion through the activation of the inositol requiring enzyme 1/X-box binding protein 1 (IRE1/XBP1) pathway. We have also shown that mRNA of the spliced, active form of XBP1 (sXPB1) is increased in hyperpolarized M2 macrophages, and that treatment with an IRE1 inhibitor led to a decrease in CCL-18. Here, we further investigated the involvement of the IRE1/XBP1 pathway in M2 macrophages through the expression of sXPB1 and various macrophage markers in archived tissues derived from patients diagnosed with fibrotic lung disease.

METHODS: Human interstitial lung disease biopsy tissues were obtained from the Surgical Lung Biopsy Cohort at St. Joseph's Healthcare Hamilton. Regions of interest (fibrotic and non-fibrotic) were identified by a pathologist through H&E stained slides. Tissue microarrays (TMAs) were created by taking cores from regions of interest in parent blocks, and inserting them into drilled host paraffin blocks. Histological assessment was conducted on the Leica Bond RX immunostainer using duplex automatic RNAscope® fluorescent in-situ hybridization (FISH) assays to detect mRNA transcripts of CCL-18 and Cluster of Differentiation (CD) 68, and Basescope™ in- situ hybridization technology to detect full length and spliced XBP1. Various immunohistochemical stains for M2 macrophage markers were conducted as well. Slides were digitalized using the Olympus VS120 Slide Scanner, and quantitative tissue analysis was performed using HALO™ Image Analysis Platform.

RESULTS: We demonstrate here that sXBP1 is expressed in higher levels in fibrotic cores from IPF patients ($p<0.005$), as demonstrated through staining with Basescope™ technology and quantification with HALO software. No significant difference was found for full-length XBP1. Duplex FISH shows colocalization of CCL-18 and CD68 mRNA transcripts in human IPF tissue. Observations of serial sections with immunohistochemical staining showed colocalization of M2 macrophage markers CD68, CD206, and Dectin 1 in fibrotic lung disease tissue.

CONCLUSIONS: Spliced XBP1, the signature of activation of the IRE1 pathway, is found at increased levels in fibrotic lung tissue, supporting its involvement of the IRE1/XBP1 pathway in human fibrotic lung disease. The colocalization of CCL-18 and CD68 mRNA confirms the ability of macrophages to produce CCL-18 in IPF, and translates our previous findings to the patient setting. Additionally, colocalization of various macrophage markers including CD68, CD206, and Dectin 1 with XBP1 and spliced XBP1 demonstrate compartmentalization to the macrophage. Overall, the detection of sXBP1 in human IPF tissues, combined with previous results in experimental systems, supports IRE1 inhibition and macrophage polarization as a potential therapeutic target in IPF.

F20.

Gla residues in MGP protect from phosphate-induced vascular calcification

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BACKGROUND: Mutations in the matrix Gla protein (MGP) gene in humans lead to Keutel syndrome, a rare autosomal recessive disorder hallmarked by cartilage and vascular calcification, midface hypoplasia and pulmonary stenosis. *MGP* is highly expressed in chondrocytes and vascular smooth muscle cells (VSMCs). *Mgp* 'knockout' (*Mgp-/-*) mice display most of the phenotypic traits of Keutel syndrome. Earlier, we demonstrated that the introduction of a mutation in *Phex* gene in *Mgp-/-* mice prevents vascular calcification. However, it was not clear whether fibroblast growth factor 23 (FGF23), a phosphate regulating hormone acting downstream of PHEX, plays a critical role in the process. MGP carries 4 γ-carboxylated glutamic acid (Gla) residues. Although these residues are thought to be critical for MGP's anti-mineralization function, so far no genetic experiment has been performed to examine their role in the vascular tissues.

AIMS:
1) To investigate the effects of FGF23 on the vascular calcification phenotype in MGP-deficient mice.
2) To investigate the anti-mineralization property of mutated MGP that lacks the Gla residues.

METHODS: We crossed *Mgp+/-* mice with the *ApoE-Fgf23* transgenic mice to generate *Mgp-/-;ApoE-Fgf23* mice. As is the case with the PHEX-deficient (*Hyp*) mice, *Mgp-/-;ApoE-Fgf23* mice show high levels of circulating FGF23 and hypophosphatemia. The vascular calcification phenotype in these mice was analyzed by microCT, histology and alizarin red staining of the thoracic aorta. For the second aim, we generated a new transgenic line (*SM22GlamutMgp*) expressing a mutant form of MGP in which the four conserved glutamic acid residues to alanine. We generated *Mgp-/-;SM22GlamutMgp* mice and examined the vascular calcification phenotype as above.

RESULTS: Alizarin red staining and histological analyses showed that unlike *Mgp-/-* mice, at 4 weeks of age, *Mgp-/-;ApoEFgf23* mice did not show any sign of vascular calcification. A high phosphorus diet induces rapid vascular calcification in these mice. The *Mgp-/-;SM22Glamut* mice did not express the endogenous MGP, but express in the VSMCs a mutant form lacking the conserved glutamic acid residues. Surprisingly, these mice never developed vascular calcification. Under hyperphosphatemic conditions, these mice develop severe arterial calcification.

CONCLUSIONS: FGF23 and Inorganic phosphate axis acts as a major regulator of vascular calcification in *Mgp-/-* mice. Here we show that the known Gla residues are dispensable for MGP's anti-mineralization function under normophosphatemic condition. This latter finding may explain why drugs that interfere with gamma carboxylation of Gla residues may result in high mortality in end stage renal disease.

F.21.

Role of DC-STAMP-mediated signaling in cell fusion and osteoclast maturation

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BACKGROUND: Osteoclasts (OC) are the bone-resorbing multinuclear cells that originate from myeloid progenitor cells through repetitive cycles of cell fusion. DC-STAMP, a transmembrane protein, is essential for cell-cell fusion and formation of fully functional OC although the molecular mechanisms that are not well understood. Utilizing RAW cell lines we earlier demonstrated that, during osteoclast (OC) differentiation, heterogeneity in membrane expression levels of DC-STAMP correlates with efficient cell fusions during the multinuclear OC formation. Optimal fusion was observed when DC-STAMPlow and DC-STAMPhigh cells interact. Herein, we examined how complete absence of DC-STAMP in the osteogenic progenitor cells (OCPs) affects their ability to participate in cell fusion events needed for efficient osteoclastogenesis and affects their ability to mount calcium (Ca2+) flux in response to an appropriate stimulus.

METHODS: We isolated bone marrow macrophages (BMMs) from wild type (WT) and DC-STAMP knockout (KO) mice. To analyze cell fusion, we labeled DC-STAMP+/+ and DC-STAMP-/- BMMs obtained from WT and KO mice respectively with red and green membrane dyes and cultured them in osteoclastogenic condition and monitored cell-cell fusion using live cell imaging. Moreover, we examined the expression dynamics and fate of DC-STAMP protein in forming OC employing the retroviral-mediated expression of GFP-tagged DC-STAMP protein in DC-STAMP-/-cells. In addition, we investigated how DCSTAMP alters expression levels of key OC-related genes in WT and KO cells. Finally, we investigated how DC-STAMP alters calcium flux during OC differentiation to compare their differential response by exposing the cells to receptor activator of nuclear factor kappa-B ligand (RANKL).

RESULTS: We find that DC-STAMP-/- BMMs are incorporated into forming OC; however, DC-STAMP+/+ cells are essential to initiate such cell fusion events. Following retroviral vector-mediated complementation of GFP-tagged DC-STAMP protein expression in DC-STAMP-/- BMMs, we noted that during OC formation, DC-STAMP expression level remained high but progressively declined during several rounds of cell-cell fusion and levels were low or absent in mature OCs. Interestingly, we noted the absence of DC-STAMP do not affect the mRNA and protein expression levels of Nuclear Factor Of Activated TCells, Cytoplasmic, Calcineurin-Dependent 1 NFATc1, or gene expression of other osteoclastic factors such as ACP5, CTSK, ATP6V0D2 but affected nuclear localization of NFATc1. We found that RANKL-induced Ca2+ oscillations were still present in DC-STAMP-/- osteoclast precursors, and increased in intensity compared to DC-STAMP+/+ OCPs during later stages of differentiation.

CONCLUSION: Our findings indicate that DC-STAMP OCP expression levels are high during the early cell-cell fusion events but progressively decline and are absent or low in mature OCs. Our results also indicate that while DC-STAMP-/- OCPs cannot form multinuclear OCs, they still can fuse with DC-STAMP +/+ OCPs and becomes part of maturing OCs. Finally, our data demonstrate even though calcium oscillations are present in DC-STAMP-/- OCPs, NFATc1 nuclear translocation is deficient, suggesting that DC-STAMP acts in NFATc1 pathway, but downstream of calcium signaling.

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Poster session on Friday (F)

Tissue Engineering/Repair/Regeneration

F22.

Dual mechanism of smart delivery biomaterials

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Current cancer therapies broadly target rapidly proliferating malignant cells while also affecting healthy cells. Thus, an area of intense research focus is developing a highly specific delivery method. We have previously reported on a pH-responsive folic acid (FA) conjugated nanopolymer that has the requisite features for efficient drug delivery. We demonstrated that this nanopolymer can effectively penetrate the inner core of spheroids (3-dimensional cancer models) and deliver anti-cancer therapies. Surprisingly, nanopolymers not loaded with chemotherapeutics also decreased spheroid volume and revealed a previously unknown intracellular mechanism of action of the nanopolymer. Literature has demonstrated that the folic acid receptor (FR) can act as a transcription factor responsible for transcribing growth promoting genes. We hypothesize that the FA-conjugated nanopolymer interacts with FR to prevent the transcription of cell proliferation proteins leading to a decrease in cancer cell proliferation. The FA-conjugated nanopolymer binds to the FR, and is transported into the cell towards the nucleus where, due to its large size, directly prevents the binding of transcription factors, decreasing cell viability. Preliminary results suggest that the empty FA-conjugtaed nanopolymer is internalized and brought into the nucleus where within 48 hours it begins to exert its ant-cancer effect. These results provide insight into the mechanism of malignant cell growth and proliferation, allowing for a new generation of treatments which will increase specificity and reduce systemic toxicity. This novel targeting delivery system may overcome many of the negative cytotoxic effects experienced by patients receiving chemotherapy. Additionally, these studies reveal the dual actions of this nanocarrier, demonstrating that this novel drug delivery system is more powerful than originally thought.

F23.

Nanoparticle-functionalized poly-methyl methacrylate bone cement for sustained chemotherapeutic delivery

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INTRODUCTION: Poly-methyl methacrylate (PMMA) bone cement is one of the most commonly used bone substitutes in orthopedic surgery. In clinical practice it can be loaded with various drugs, such as antibiotics or anti-cancer therapeutics, as a means of local drug delivery. However, studies have shown that drugs loaded into PMMA cement tend to release in small bursts in the first 48-72 hours, and the remaining drug is trapped without any significant release over time. The objective of this study is to develop a nanoparticle-functionalised PMMA cement for use as a sustained doxorubicin delivery device. We hypothesise that PMMA cement containing mesoporous silica nanoparticles will release more doxorubicin than regular PMMA.

METHODS: High viscosity SmartSet ™ PMMA cement by DePuy Synthes was used in this study. The experimental group consisted of 4 replicates each containing 0.24 g of mesoporous silica nanoparticles, 1.76 g of cement powder, 1ml of liquid cement monomer and 0.1 mg of doxorubicin. The control group consisted 4 replicates each containing 2.0 g of cement powder, 1ml of liquid cement monomer and 0.1 mg of doxorubicin. Each replicate was cast into a cylindrical block, and then incubated in a phosphate-buffered saline solution which was changed daily for 21 days. The concentration of eluted doxorubicin in each solution was then measured using a fluorescent plate reader against a doxorubicin standard curve.

RESULTS: Each cement block weighed 2.47 ± 0.18 g representing 84.96 ± 5.97% of the initial mixture weight. The experimental group contained an average of 8.28 ± 0.09 % (W/W) mesoporous silica nanoparticles. The experimental group released 31.39 ± 10.62 % of the initially loaded doxorubicin which was more than the 21.35 ± 0.21 % released by the control group, however this trend was only approaching statistical significance p 0.08.

CONCLUSION: The use of mesoporous silica nanoparticles to improve drug release from PMMA cement shows promise. Further optimisation of the experimental protocol are needed to reduce the large variability in the rate of drug release from mesoporous silica nanoparticles loaded cement. Furthermore, *in vitro* and *in vivo* experiments are required to test the efficacy of released doxorubicin on tumour cell growth.

F24.

Development of a 3D tissue mimetic of shoulder joint infections with Cutibacterium acnes

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INTRODUCTION: Periprosthetic joint infection (PJI) is a devastating and costly post-surgical complication resulting in connective tissue necrosis and implant loosening. *Cutibacterium acnes*, a skin commensal but opportunistic anaerobic pathogen, causes ~60% of post-surgical PJIs in the shoulder by forming antimicrobial-resistant biofilms on implant surfaces. Due to the lack of reproducible models in which to study PJIs, the molecular mechanisms regulating *C. acnes* biofilm formation on implants and the impact of these biofilms on shoulder connective tissue repair and homeostasis are poorly understood. To address this problem, we have developed a 3D shoulder-joint implant mimetic (S-JIM) of post-surgical PJIs to investigate the interactions between *C. acnes* biofilms and shoulder connective tissue and to identify novel targets for clinical intervention.

METHODS: The S-JIM is an *in vitro* co-culture system of *C. acnes* and primary human rotator cuff (HRC) cells. HRC cells were derived from connective tissue offcuts from patients undergoing shoulder arthroplasties (HSREB #104888). *C. acnes* and HRC cells were distributed into a collagen-impregnated cellulose scaffold strip and wrapped around a mandrel core in several layers to generate a cylindrical 3D "tissue mimetic". This biocomposite was incubated under 5% pO2 in high glucose DMEM with 0.4% soytone to establish an oxygen and nutrient accessibility gradient across the exterior and interior "tissue" layers. At various timepoints post-incubation, the scaffold strip was unrolled, sectioned by circumferential layer, and subjected to cell staining, EF5-based hypoxia analyses and quantitative PCR analyses of gene expression to assess for cellular viability and responses to culture conditions.

RESULTS: Confocal Z-stack analyses indicated that HRC cells were viable in all S-JIM layers under 5% pO2 for at least 10 days in the absence of *C. acnes*. The expression levels of *HMOX1* (encoding Heme Oxygenase 1) and *BAX:BCL2* (encoding Bcll2 Associated X and B-Cell Lymphoma 2) were similar across all S-JIM layers, indicating a lack of oxidative stress and apoptosis respectively. An ~3-fold increase in *HIF1A* expression (encoding Hypoxia Inducible Factor 1a) and an EF5 fluorescence gradient in HRC cells in the inner scaffold layers relative to the outer layers was consistent with cellular responses to an increasing O2 gradient. Co-culture assays showed adherence-like interactions resulting in HRC cell lysis after 6 hours under 2% pO2.

DISCUSSION: To our knowledge, the S-JIM is the first 3D connective tissue mimetic of PJIs. Our findings indicate that S-JIMs can generate an anaerobic core that is favorable for *C. acnes* proliferation and can support *C. acnes* and HRC in co-culture. Major anticipated advantages of S-JIMs over 2D cell culture and animal models include the ability to directly and reproducibly assess the impact of *C. acnes* on shoulder connective tissue and to investigate the molecular mechanisms regulating *C. acnes* biofilm formation within a microenvironment relevant to human physiology. We anticipate using S-JIMs to identify novel approaches of preventing *C. acnes* biofilm formation on implants, such as pre-mature quorum induction and surface conditioning with DNAses. In summary, S-JIMs have the potential to provide reproducible, human cell-based evidence for novel therapeutic approaches to prevent *C. acnes* PJIs.

F25.

The effect of sLink N on hypertrophic chondrogenesis of BM-MSCs

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BACKGROUND: Osteoarthritis (OA) is a chronic debilitating disease affecting millions of people worldwide. Although OA affects the whole joint, it is typically characterized as a degenerative articular cartilage disease. Cartilage degeneration is often described as an imbalance in the anabolic and catabolic activities of chondrocytes; however, other factors such as increased apoptosis and mechanical wear are also involved. Current treatment options are targeted to symptomatic relief rather than the underlining pathology. The reason for this is because there are no effective therapies that can ultimately reverse or repair the lost or defective cartilage. As a result, when a patient's mobility is compromised and pain becomes unbearable, arthroplasty is performed. Bone Marrow Mesenchymal Stem Cells (BM-MSCs) offer a potential solution as a cartilage repair strategy, since they are capable of differentiating into chondrocytes and potentially replenishing damaged or lost cartilage. However, one of the main disadvantages in using BM-MSCs as a repair strategy is that they selectively differentiate into hyperchondrocytes, expressing type 10 collagen (COL X) and undergoing endochondralossification. Notwithstanding, BMMSCs isolated from OA patients often express elevated levels of COL X, rendering them even more susceptible to ossification. Link N (DHLSDNYTLDHDRAIH), a growth-like factor and cleavage product of Link protein, has been demonstrated to induce both intervertebral disc and cartilage repair both *in vitro* and *in vivo*. In addition, Link N has been shown to decrease the expression of ColX in OA BM-MSCs, and enhance MSCs chondrogenic differentiation *in vitro*. However, its effects on endochondrogenic versus chondrogenic differentiation of BM-MSCs remains unknown. A shorter and more potent version of Link N, sLink N, has recently been described. In this study we characterize the effects of sLink N on COL X expression in OA BM-MSCs, and determine whether pretreatment of sLink N can mitigate hypertrophic chondrogenesis.

METHODS: OA BM-MSCs were isolated from donors undergoing total hip replacement surgery. MSCs were verified by Flow cytometry and their ability to differentiate into chondrocytes, osteocytes, and adipocytes in culture. Cells were expanded and divided into two groups: sLink-N treated and non-treated. Proliferation rates, gene expression (COL X, COL II, Aggrecan, SOX-5, -6, -9, RUNX2, ALP), and chondrogenic differentiation were evaluated.

RESULTS: OA BM-MSC doubling rates were significantly decreased following sLink-N treatment. Flow cytometry demonstrated MSC markers were maintained beyond 10 passages when compared to non-treated cells. Gene expression analysis and chondrogenic differentiation experiments are being evaluated.

CONCLUSION: Pretreatment of BM-MSCs with sLink N may enhance their chondrogenic potential for cartilage repair by decreasing hyperchondrogenic differentiation.

F26.

Localized doxorubicin delivery using 3D printed porous scaffolds inside a bioprinted bone-like invitro 3D model

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INTRODUCTION: Chemotherapy is an important treatment modality for patients with spinal metastases, with Doxorubicin (Dox) being one of the most effective options. The high systemic doses required for the drug to be effective prevent its prolonged use due to the significant heart failure it causes with cumulative doses. Our lab has previously shown that 3Dprinted porous polymer scaffolds are as effective as direct treatment for local delivery of Dox to inhibit bone metastasis cancer cell growth in a 2D culture. However, the effectiveness of this drug delivery system remains to be tested in a model that resembles the *in-vivo* environment. Bioprinting is an effective tool for creating tissue-like 3-dimensional tumor models for therapeutic screening. Using this technology, our lab is designing a bone-like 3D model for testing the effectiveness of 3D-printed porous polymer scaffolds for drug delivery to cancer cell lines and patient derived cancer cells of breast, lung and prostate origin.

METHODS: MDAMB-231 (breast), HCC-827 (lung) and LAPC4 (prostate) cancer cell lines were obtained commercially and 3 different patient derived spine metastasis cell samples per tumor type were obtained from patients participating in this study that underwent tumor resection at the Montreal General Hospital. Primary osteoblasts were isolated from vertebral bodies of organs donors through collaboration with Transplant Quebec. PORO-LAY polymers were 3D printed into porous scaffolds and loaded with 1-3 µg of Dox, for which we have previously determined release kinetics. Tumor or osteoblast cells were fluorescence stained and 3-dimensional co-culture tumor models comprising of an inner tumor core and an outer osteoblast cell perimeter were printed using the CELLINK BIO X bioprinter with a 1% alginate / 7% gelatin bioink in 24 well plates and crosslinked with calcium chloride. 3D printed scaffolds loaded with or without drug were inserted inside the tumor core and plates were incubated for 3 weeks in 10% serum media. Images were captured with an inverted fluorescence microscope every 3 days to assess for migration between the 2 cell compartments. Cell viability and metabolic activity were conducted using the LIVE/DEAD and alamarBlue assay.

RESULTS: To date, we have shown that bioprinted 3D cultures allow for sustained cell viability over a 4-week period. We expect the Dox loaded 3D printed scaffolds will decrease the migration of tumor cells into the surrounding stroma, decrease metabolic activity of 3D printed tumor cells, and decrease the viability of tumor cells more than direct Dox treatment or control.

CONCLUSION: Use of 3D printed porous scaffolds for targeted drug delivery may allow for effective inhibition of tumor recurrence after resection in patients with metastatic spine disease by targeting the full effect of the drug at the site of the tumor and sparing the rest of the body from side effects that normally arise with systemic treatment. The use of bioprinting in these experiments showcased the potential for this technology for creating physiological screening models with greater resemblance to tissue microenvironments. Future use of these models can identify appropriate therapeutic dosage and facilitate rapid and more effective animal studies.

F27.

A method for studying MLO-Y4 osteocyte response to simulated microgravity in embedded 3D collagen droplet scaffolds

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The limited number of opportunities for biological research in space has generated the need for groundbased microgravity simulators. The Rotary Cell Culture System (RCCS) has become one of the most widely used devices to simulate microgravity conditions in cultured cells. Nevertheless, the device is known to produce differing effects depending on the cell type, microcarriers used, speed of rotation, vessel dimensions and laboratory conditions. Osteocytes are the mechanosensors of bone, producing biological signals when mechanical forces are applied or removed from their environment. It is thought that these cells play an important role in astronaut osteoporosis, however the cellular mechanisms of the disease have yet to be elucidated. We sought to characterize the response of MLO-Y4 osteocytes in the device using 3D gel scaffolds composed of collagen I and hydroxyapatite nanoparticles to produce a bone-like environment for the cells. The gel was inoculated with a cell suspension and mixed to ensure homogenous cell distribution. The mixture was then formed into droplets to allow compatibility with the RCCS and to ensure good nutrient diffusion which would otherwise be hindered in a larger construct. The density of the scaffold was optimized such that the droplets were stably suspended in the rotating culture medium using a precise concentration of hydroxyapatite. The density of cells in the scaffold was also optimized to mimic the intricate network of the lacuno-canalicular system which osteocytes form in bone tissue. Osteocyte markers of mechanical stimulation were studied by quantitative real-time PCR to assess the response of MLO-Y4 osteocytes to the simulated microgravity environment. Our future work will utilize our optimized simulated microgravity environment in the RCCS to conduct a transcriptome analysis of MLO-Y4 osteocytes which will be compared to a static control regime and a mechanical loading regime. From these data, we will determine novel genes affected by mechanical unloading in osteocytes which can be further validated in true microgravity spaceflight. This work therefore marks an important first step in determining potential therapeutic targets to combat astronaut bone loss.

F28.

Characterization of microvasculature in Duchenne muscular dystrophy

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BACKGROUND: Duchenne muscular dystrophy (DMD) is a x-linked recessive neuromuscular disease characterized by progressive muscular degeneration. It is caused by dysfunctional dystrophin leading to contraction-induced damage, necrosis, and respiratory impairment and cardiomyopathy fatalities. There is currently no cure. Angiogenesis, the process of new blood vessel formation from preexisting ones, has been shown to be dysfunctional in DMD mice models and DMD patients, leading to pathologic ischemia, inflammation, and ultimately fibrosis. Recent studies have shown that vascular endothelial growth factor (VEGF) administration can induce neo-angiogenesis, however, it did not improve perfusion, nor did it reduce inflammation and fibrosis. Our lab has shown that angiopoietin-1 (Ang-1), a pericyte angiogenic factor, alone is capable of restoring angiogenic function. However, endogenous levels of Ang-1 has been shown to be significantly lower in our severely fibrotic mouse model tissue of DMD than in less-affected muscle tissue of these mice and in healthy wild type mice. Moreover, the expression of Tie 2, a receptor tyrosine kinase expressed on endothelial cells and pericytes and the receptor for Ang-1 has not been characterized in DMD mice models nor DMD patients. Lastly, while the interaction between Ang-1/Tie-2 interaction is shown to be anti-inflammatory and angiogenic, the Ang-2/Tie-2 antagonistic interaction is pro-inflammatory.

HYPOTHESIS AND OBJECTIVE: We hypothesize elevated pro-inflammatory factors in DMD skeletal muscle results in abnormal Angiopoietin/Tie-2 signaling, shifting from an Ang-1/Tie-2 interaction to Ang-2/Tie-2 interaction as disease severity progresses. Overarching Goal of this project is to characterize abnormal vasculature in DMD in detail.

MATERIALS AND METHODS:
Specific Aim 1: Characterize changes in Angiopoietin-1, Angiopoietin-2, Tie-2, and phosphorylated Tie-2 expression in muscle tissue from preclinical mouse models of DMD.
Muscle tissues (gastrocnemius and diaphragm) from each of our 3 DMD mouse models that display weak (mdx), intermediate (mdx:utrn+/-) and severe (mdx:utrn-/-) disease phenotypes, will be collected at several time points prior to their development of overt fibrosis and after the onset of muscle fibrosis. Immunoblots will be used to assess the expression of Angiopoietin/Tie-2 factors, pericyte markers (e.g. PDGFRβ, NG2, αSMA), secreted proinflammatory markers (IL-6, IL-1, TNFa), and endothelial inflammatory markers (e.g. E-selectin, CD31, isolectin). H&E and Masson's Trichome staining will be performed on each of these tissues to assess the extent of muscle degeneration/regeneration (percentage of centrallynucleated myofibers) and fibrosis, respectively.
Specific Aim 2: Evaluate alterations to muscle vasculature and fibrosis in response to pro-inflammatory factors secreted from DMD affected skeletal muscles.
Primary fibroblast and endothelial cell cultures from previously mentioned muscle types will be subjected to exogenous proinflammatory cytokine mixes. Western blots and immunocytochemistry will be used to assess gene expression alterations within the cell cultures, mainly fibrotic factors and endothelial inflammatory markers.

DISCUSSION: Our lab is currently working with Akrivis LCC in developing a vascular-targeted therapy. Thus, characterization of the vasculature will enable us to target inflammatory biomarkers and evaluate an optimal window of opportunity for drug delivery. Allowing for reductions in inflammation, ischemia, and fibrosis in the microvasculature and allow for endogenous muscle repair and the benefits of other therapeutics to be fully realized.

F29.

A new mechanism of latent TGF-beta1 presentation by lung macrophages

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BACKGROUND: Accumulation of scar tissue in fibrosis diminishes organ function. Fibrosis is characterized by the chronic coexistence of macrophages (MΦ), which produce pro-fibrotic growth factors and myofibroblasts (MFs), which secrete and contract collagen. Our recent data show that MΦ express latent TGF-β1, which is only activated in co-culture with MFs upon direct contact. Here we investigate the mechanism of how MΦ present latent TGF-β1 to MFs for local activation. Regulatory T cells are known to extracellularly present latent TGF-β1 using the transmembrane protein glycoprotein A repetitions predominant (GARP).

METHODS: To test whether tissue MΦ express GARP, we immuno-colocalized GARP with MΦ marker CD68 in normal, inflamed and fibrotic human lung. Primary cultures of human MΦ at different polarization states were tested for GARP expression by Western blotting, flow cytometry and RT-PCR.

RESULTS: Our results show that GARP is expressed in MΦ of normal (n=17) and inflamed lung (n=32) and on the surface of *in vitro* polarized pro-inflammatory MΦ (6%). Western blots performed with cultured MΦ under non-reducing conditions detect GARP, LAP and TGF-β1 at 250 kDa, identifying the GARP-LAP-TGF-β1 complex. To test whether MFs release latent TGF-β1 from MΦ-GARP, we measured active TGF-β1 levels in MF co-cultures with GARP-expressing and -depleted MΦ. The release mechanism is currently under investigation.

CONCLUSIONS: Collectively, our results indicate that GARP serves as novel surface anchor on MΦ to provide a local TGF-β1 source in inflammation and fibrosis. Interfering with local TGF-β1 presentation and/or activation are potential novel antifibrotic strategies.

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